counterpart of B cells at the midstage of differentiation. Ten to 20 percent of cases are T cell lineage; 2% are derived from the monocyte-myeloid lineage. Recently, clonal rearrangement of the Tβ receptor has been described in patients with T-derived non-Hodgkin's lymphoma.[172,173]

Malignant lymphoma, lymphoblastic. Malignant lymphoma, lymphoblastic, or lymphoblastic lymphoma, is a high-grade malignancy. The nuclear membrane is characteristically deeply subdivided, exhibiting either a lobulated (convoluted) appearance or a fine linear (nonconvoluted) subdivision in a round nucleus. Lymphoblastic lymphoma represents approximately one-third of the cases of non-Hodgkin's lymphomas in children and 5% of cases in adults. The disease is more prevalent in males; these patients often have a mediastinal mass. In some cases, the disease may evolve into a leukemic phase morphologically indistinguishable from T-ALL. The malignant cells are T cells, form E rosettes, react with T cell antisera,[223–225] and have rearrangements of the Tβ receptor.[226] Studies with monoclonal antibodies have demonstrated marked heterogeneity. Lymphoblastic lymphoma cells differ from T-ALL in that the cells rarely express the surface markers common to immature thymocytes (group I);[227] phenotypes are equally divided between group II and group III T-ALL. In 40% of cases, the cells are reported to express CALLA; CALLA expression is less common in T-ALL (10%).[218]

Malignant lymphoma, small noncleaved cell. Malignant lymphoma, small noncleaved cell includes Burkitt's lymphoma and other lymphomas previously designated undifferentiated non-Burkitt type (high grade). Burkitt cells from peripheral blood and bone marrow are usually classified as L3 by the FAB criteria.[185,186] Most cases of Burkitt's lymphoma from Africa are endemic and are associated with the Epstein-Barr virus (EBV). Most non-African cases (nonendemic) are EBV negative.[228] Chromosomal abnormalities involving chromosome 8 (carrying the oncogene c-*myc*) and either 2, 14, or 22 occur in virtually all cases of endemic and nonendemic Burkitt's lymphoma.[229] These are designated t(2;8), t(8;14) and t(8;22), respectively. Usually the light chain class expressed on these cells is correlated with the translocation, ie, κ in t(2;8) and λ in t(8;22). African Burkitt's lymphoma cells have receptors for C'3 and for the Fc portion of IgG in addition to the EBV receptor. American Burkitt's lymphoma cells do not express these receptors.[228] Phenotyping of cell lines derived from patients with undifferentiated lymphoma of the Burkitt's and non-Burkitt's type have demonstrated heterogeneity.[230] These studies suggest that Burkitt cells follow a divergent pathway of B cell evolution because they are all TdT negative (unlike early B cell non-T-ALL). The most primitive of the Burkitt cell lines are Ia and B1 positive and may or may not express CALLA. Maturation was evident in other Burkitt cell lines by the expression of Cμ, surface membrane IgM, and/or IgM secretion. Some of these Burkitt cell lines also expressed the Tac antigen.

Peripheral T cell lymphoma. Peripheral T cell lymphoma would usually be classified as malignant lymphoma, large cell immunoblastic (high grade) under the working formulation. However, this tumor has unique features and will be described separately. The term "peripheral T cell lymphoma" is used to distinguish it from lymphoblastic lymphoma of presumed thymic origin. Peripheral T cell lymphomas are thought to derive from peripheral T lymphocytes in lymph nodes and other nonlymphoid sites. These lymphomas comprise a broad spectrum of morphologic types of lymphocytes. In all instances, the cells have T cell markers admixed with epithelioid histiocytes, plasma cells, eosinophils, and vascular hypertrophy. Clinically, peripheral T cell lymphoma is characterized by generalized lymphadenopathy, weight loss, and a high incidence of pulmonary involvement.[231] Surface markers are usually but not always characteristic of mature T helper cells,[232] including the T4/Leu-3 helper-associated antigen and the T3/Leu-4, T11/Leu-5, and T1/Leu-1 pan-T antigens. Rearrangement of the Tβ receptor has been reported.[226]

Tγ lymphoproliferative disease. Tγ lymphocytes are a subset of T lymphocytes with receptors for the Fc portion of IgG. A high proportion of normal Tγ lymphocytes are LGL. These cells are thought to be responsible for natural killer (NK) and antibody-dependent cell-mediated cytotoxicity in humans[233] and rodents.[234] A lymphoproliferative disorder made up of predominantly Tγ lymphocytes has been described; we refer to this as chronic Tγ lymphoproliferative disease.[235] Typically, patients are elderly males with increased Tγ lymphocytes infiltrating the bone marrow and spleen.[235,236] Although the disease is not rapidly progressive, neutropenia and recurrent infections are common. Most patients do not require chemotherapy. Variants of this disease, including a more aggressive form, have been described.[237] Clonal chromosomal abnormalities,[238] as well as clonal rearrangement of the Tβ receptor, have been reported.[175,239] Cells from chronic Tγ lymphoproliferative disease usually contain acid phosphatase and β-glucuronidase and express the pan-T antigens T3/Leu-4, T11/Leu-5, the suppressor-associated antigens T8/Leu-2, and the NK-associated antigen Leu-7 (HNK-1). Other monoclonal antibodies that react with LGL[240,241] may also prove to be useful.

Cutaneous T cell lymphoma (mycosis fungoides, Sézary cell leukemia). Skin lesions are the most prominent feature of patients with cutaneous T cell lymphoma.[242] Lesions vary from limited plaques to diffuse generalized plaques, tumors, and generalized erythroderma. Rare patients with limited plaque disease and <50% with generalized plaques and tumors have extracutaneous disease detected by light microscopy evaluation of peripheral blood and lymph nodes. Special studies including cytogenetic analysis and electron microscopy indicate blood involvement in >50% of patients with limited plaque disease and most patients with generalized plaques and skin tumors.[243] Analysis of the Tβ receptor rearrangement will likely reveal a higher proportion of cases with nonmalignant cells in the blood and lymph nodes.

The malignant cells in this disorder are characterized by a cerebriform nucleus. In the skin, the cells are referred to as mycosis fungoides cells and in the peripheral blood as Sézary cells. Sézary and mycosis cells form E rosettes, react with T

antisera and anti-T monoclonal antibodies,[244,245] and have clonal rearrangements of the Tβ receptor.[173,175,226,246] In most cases, the cells express the phenotype associated with normal helper/inducer T lymphocytes (T-1/Leu-1, T3/Leu-4, T4/Leu-3 positive)[247-251] and function as helper T lymphocytes in in vitro assays.[252] The 3A1 antibody (CD7), which reacts with >85% of normal circulating T lymphocytes and with mycosis cells in the skin, does not generally react with Sézary cells in the blood.[253]

Adult T cell leukemia/lymphoma. Adult T cell leukemia/lymphoma is associated with a human retrovirus designated human T cell leukemia/lymphoma virus-1 (HTLV-1).[254,255] Virtually all patients tested have antibodies to HTLV-1.[256] Patients with this disease have been identified primarily in Japan, the United States, and the Caribbean. In the United States, the patients are young (median age 33 years), predominantly black, and born in the southeast.[253] Common clinical features include a rapid onset of symptoms with rapidly progressive cutaneous lesions and hypercalcemia. Skin lesions are variable and include small and large discrete or confluent nodules, or nonspecific plaques, papules, or patches. Patients have increased bony turnover with abnormal bone scans and elevated alkaline phosphatase and may have lytic bone lesions.[257] Lymphocytosis is common, and circulating malignant cells are present in low numbers in most patients. Peripheral lymphadenopathy is common, with retroperitoneal and hilar involvement in approximately 50% of cases. Bone marrow, gastrointestinal, pulmonary, leptomeningeal, and hepatic involvement are somewhat less common (20% to 50%). Response to combination chemotherapy is prompt and often complete, but duration of response is short (median 13 months). Opportunistic infections are extremely common in these patients.

The typical malignant circulating cells have moderately condensed nuclear chromatin, inconspicuous nucleoli, and a markedly irregular nuclear contour in which the nucleus is divided into several lobes.[258] These cells typically express the phenotype of helper/inducer T lymphocytes,[259-261] and the Tac antigen (CD25) that identifies the IL-2 receptor.[262] Variability in the expression of T3, T11, and T12 have been reported.[261] Clonal rearrangements of the Tβ receptor are identified in cells from patients with adult T cell leukemia/lymphoma.[173,175,226,239] The leukemic cells are reported to suppress B cell Ig secretion[263] by a complex mechanism involving induction of suppressor cells following activation of normal suppressor cell precursors.[261]

CLL and prolymphocytic leukemia. CLL is a monoclonal proliferation of SmIg-positive B lymphocytes.[264,265] Clonality of CLL has been demonstrated by expression of a single Ig light chain, either κ or λ, on the cell surface membrane.[266,267] More sophisticated techniques have confirmed clonality by showing unique immunoglobulin idiotype specificities,[268] a single pattern of glucose-6-phosphate dehydrogenase activity,[269,270] clonal chromosome abnormalities,[271] or immunoglobulin gene rearrangement,[157] The malignant B cell involved in CLL is an intermediately differentiated cell. The cell appears frozen in differentiation and does not mature to the final stage of B cell development, the mature plasma cell. However, recent data have demonstrated that in vitro treatment of these cells with phorbol esters or pokeweed mitogen can induce differentiation into mature immunoglobulin-secreting plasma cells.[272] In another study, this immunoglobulin secretion was preceded by a rapid increase in the level of mRNA coding for IgM, a predominantly secretory form of mRNA rather than a membrane form of mRNA.[273] This selection is similar to that seen in plasma cells, and the study clearly demonstrated at the molecular level that CLL cells consistently retain the capacity to differentiate to plasma cells and secrete immunoglobulin. Under certain circumstances, CLL cells stimulated in vitro with phorbol esters differentiate into cells with cytoplasmic protrusions and other characteristics of hairy cell leukemia.[274]

The B lymphocyte characteristic of CLL displays a relatively small amount of SmIg, estimated to be ~9,000 molecules per cell.[275] Relatively weak fluorescence of SmIg has been used to distinguish CLL from the leukemic phase of nodular and diffuse lymphocytic lymphomas and from prolymphocytic leukemia in which the cells generally display considerably more SmIg.[276,277] Immunoglobulin isotype analyses indicate that most CLL display a single heavy chain class; typically, μ or μ and δ. Less commonly, γ, α, or no heavy chain determinant is found. CLL cells display either κ or λ light chains but never both. Some data suggest that heavy chain switching can occur in B-CLL, which may indicate increasing maturity of the malignant cell.[277] Other studies indicate that CLL cells contain only μ or μ and δ and that γ is extrinsic and not synthesized by the leukemic cells.[278] Although there has been controversy as to whether CLL B cells contain CIg, the presence of cytoplasmic heavy chains (μ and δ) has been reported in most patients with CLL; no γ or α chains were detected.[279] B-CLL cells display receptors for mouse erythrocytes, a feature characteristic of immature B lymphocytes.[280] The cells also have the receptor for the Fc portion of IgG and complement with a relative increase of C'3d receptors (CR2) over C'3b receptors (CR1); this is typical of immature B cells.[281] B-CLL cells display several antigens, including Ia and human B cell antigens such as BA1, B1, B2, and B4. One unanticipated finding was that B-CLL cells display a 65-kd glycoprotein antigen previously thought to be restricted to T lymphocytes. This antigen was first recognized by using heteroantisera[282]; later, it was recognized with the T101 and equivalent monoclonal antibodies.[31-33] The precise meaning of this anomalous expression of a T cell antigen is unclear, although a normal B cell counterpart has been reported in human tonsil lymph nodes,[283] and stimulation in vitro of normal B cells with phorbol ester may induce expression of this antigen.[284] Recently, the TQ1 antigen, reported to define the inducer of suppression within the T helper subset, was identified on 60 of 75 B-CLL patients' cells.[285]

Rearrangement of immunoglobulin heavy and light chains has been reported as expected in B-CLL cells; however, rearrangement of the Tβ receptor has also been reported in ~10% of cases of B-CLL.[175] This is analogous to the reported Tβ rearrangement in 25% of non-T (pre-B) ALL, and again emphasizes that immunoglobulin and Tβ receptor rearrangement alone are not adequate to assign lineage.

In 3% to 10% of patients with CLL, the disease may evolve

into a diffuse histiocytic lymphoma (Richter's syndrome). This is associated with loss of the TQ1 antigen.[285] Most data suggest that this evolution involves transformed follicular center B cells rather than histiocytes or macrophages. Some transformations represent evolution of the malignant clone with expression of the same monoclonal immunoglobulin and karyotypic abnormality present in the original CLL clone.[286] In other cases, the lymphoma cells have different markers and immunoglobulin gene rearrangements than those of the original CLL cells; these cases probably represent the concomitant development of a B cell lymphoma or a histiocytic malignancy in patients with CLL.[287,288]

Prolymphocytic leukemia (PL) is related to CLL and is also likely to be derived from cells from the medullary cords of the lymph node. Immunoglobulin gene rearrangements of heavy and light chains have been reported.[289] Patients with PL generally have extremely high blast counts and splenomegaly but lack significant lymphadenopathy. Prolymphoblasts likely are activated cells and appear morphologically immature, with a fine lacy nuclear chromatin and one to two nucleoli; they may contain intracytoplasmic granules. These cells generally have higher density SmIg than do CLL cells; they have Ia and B4 antigens and may form rosettes with mouse erythrocytes.[220] PL cells from 14 consecutive patients reacted with the FMC7 monoclonal antibody that recognizes an antigen found on one half of normal B lymphocytes, whereas cells from only 5 of 20 patients with CLL reacted with this antibody.[7]

Approximately 5% of cases of CLL and PL result in a malignant proliferation of T rather than B cells. These cells react with T antisera and anti-T monoclonal antibodies reflecting the phenotypes of mature T lymphocytes; they lack SmIg and other B cell markers.[290,291] Many of these patients have diffuse organ and skin involvement.[290] T-CLL cells have been reported to have either helper or suppressor surface markers.[202,292,293] One patient's cells rosetted with sheep erythrocytes expressed the Leu-2 (suppressor-associated) antigen and also had SmIg and CIg (IgMλ).[294] Other instances in which the leukemia/lymphoma cells expressed characteristic features of both B and T lymphocytes have also been reported.[295]

Hairy cell leukemia. Hairy cell leukemia (leukemic reticuloendotheliosis) is characterized by invasion of the bone marrow and spleen by morphologically distinct mononuclear cells with "hairy" cytoplasmic projections.[296] These cells usually contain an isoenzyme of acid phosphatase (isoenzyme 5) that is resistant to tartrate; this isoenzyme is not unique to hairy cells. Surface markers of hairy cells are most consistent with a monoclonal proliferation of B lymphocytes.[297-300] SmIg with a single light chain is frequently identified,[299,300] as are B cell-associated antigens including Ia, B1, FMC-1, FMC-7, and sometimes BA-1.[299-301] The PCA-1 antigen (but not the PC-1 antigen) typically on plasma cells is identified on hairy cells; these data suggest that hairy cells may be pre-plasma cells.[302] Perhaps the most convincing evidence for the B cell origin of hairy cells comes from studies of immunoglobulin genes which indicate clonal rearrangement of heavy chain genes and at least one light chain gene.[303,304] Most cases of hairy cell leukemia demonstrate the 53-kd to 57-kd Tac antigen (IL-2 receptor) typically identified on select T cell malignancies and activated T cells.[304] Another antigen with a mol wt of 52 to 67 kd, designated HC-2, appears to be restricted to hairy cell leukemia cells.[305,306] The αS-HCL 1 antibody reacts with normal B lymphocytes and B cell malignancies, including hairy cell leukemia.[109] The αS-HCL 3 reacts with normal monocytes and AML cells but is restricted to hairy cell leukemia among the lymphoid malignancies.[109] Approximately 2% to 3% of normal peripheral blood B lymphocytes express this antigen; these appear to be activated B cells.

Myeloma and related disorders. The malignant B cells of Waldenström's macroglobulinemia, heavy chain disease, and multiple myeloma represent a further step in the maturation of medullary cord B cells.[264] Like CLL cells, cells from patients with Waldenström's macroglobulinemia express SmIg and Ia, B1, and B4 antigens.[11] Unlike CLL cells, however, these cells express the PCA-1 antigen but do not express the B2 antigen nor do they rosette with mouse erythrocytes.[4,220] The plasma cell and its malignant counterpart, the myeloma cell, represent the most differentiated B lymphocytes. These cells synthesize large quantities of immunoglobulin and have CIg, but usually lack SmIg and the Ia, B1, B2, and B4 antigens.[307] Plasma cells and myeloma cells, like other mature B lymphocytes, usually lack CALLA, but a recent study has suggested that rare cases of CALLA-positive myeloma represent an aggressive subtype with a poor prognosis.[308] Plasma cells and myeloma cells stain intensely with the OKT10 monoclonal antibody as well as the anti-PCA-1 and anti-PC-1 antibodies.[309,310]

CORRELATES OF CELLULAR DIFFERENTIATION WITH LYMPHOID MALIGNANCIES

Substantial data suggest that the phenotypes of most leukemia cells are not unique but reflect characteristics of normal cells. None of the surface markers we have reviewed are leukemia specific; all can be identified on normal as well as malignant cells. Most of the monocyte, granulocyte, and lymphocyte antigens are found on mature and immature cells. However, CALLA, BA-2, and RFB-1 are expressed primarily on immature bone marrow cells. This observation is consistent with the phenotypes of leukemic cells, since the CALLA and BA-2 antigens are present on primitive leukemia cells (ALL and lymphoid blast crisis of chronic myelogenous leukemia cells) but only rarely on more mature leukemia or lymphoma cells. Distribution of the reactivity of the monoclonal T antibodies is likewise consistent with this hypothesis. The most primitive thymocyte markers, OKT9 and OKT10, are found on most T-ALL cells, whereas T3/Leu-4, T8/Leu-2, and T4/Leu-3, which are found on mature thymocytes and circulating T lymphocytes, are more often identified on more mature T cell leukemias.

A proposed scheme of normal lymphoid differentiation is presented in Fig 2. This scheme is based on the concept that the phenotype of normal lymphoid cells at each level of differentiation can be detected from the phenotype of its malignant counterpart. Although some malignant cells may have an aberrant phenotype, the data presented suggest that

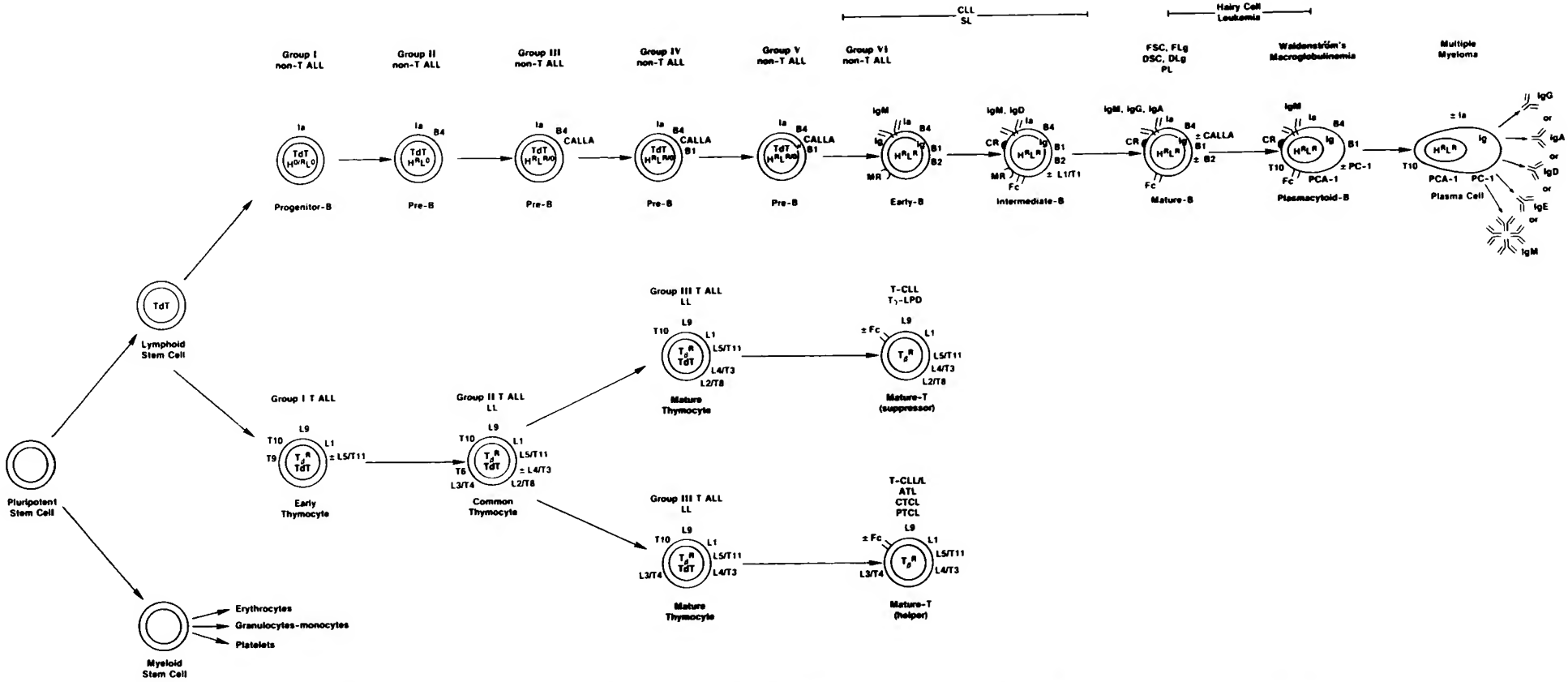

Fig 2. Schematic representation of human lymphoid differentiation and related lymphoid malignancies. ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; SL, malignant lymphoma, small lymphocytic; FSC, malignant lymphoma, follicular small cleaved cell; FLg, malignant lymphoma, follicular large cell; DSC, malignant lymphoma, diffuse small cleaved cell; DLg, malignant lymphoma, diffuse large cell; PL, prolymphocytic leukemia; LL, lymphoblastic lymphoma; Tγ-LPD, Tγ-lymphoproliferative disease; ATL, adult T cell leukemia/lymphoma; CTCL, cutaneous T cell lymphoma; PTCL, peripheral T cell lymphoma; TdT, terminal deoxynucleotidyl transferase; H, heavy chain; L, light chain; O, germline configuration; R, rearranged gene; T^R_β, clonal rearrangement of the Tβ receptor; MR, mouse rosette; CR, complement receptor.

most malignant lymphoid cells reflect the phenotype of a normal lymphocyte. The proposed phenotype of the progenitor B lymphocyte probably has the same surface markers as the group I non-T-ALL and represents the earliest identifiable B cell. This cell expresses the Ia antigen, but no other B cell-associated antigens. The next level of B cell differentiation coincides with the group II non-T-ALL; heavy but not light chain immunoglobulin genes are rearranged. At the next level of B cell differentiation, the cells express CALLA and light chain gene rearrangements occur; this coincides with group III non-T-ALL. With sequential steps in B cell differentiation, the B1 antigen is expressed, followed by $C\mu$ and then SmIg. At the next level of B cell differentiation, the B cell acquires the B2 antigen and the receptor for mouse erythrocytes; both SmIg and CIg are present. Most CLL cells—and malignant lymphoma, small lymphocytic type cells—express the phenotype of intermediate B lymphocytes. The cells express receptors for complement and the Fc portion of IgG, Leu-1, in addition to the surface markers identified on more primitive B cells. At this level of differentiation, there is low-density SmIg. The maturing B cells express high-density SmIg (IgM, IgG, or IgA) without B2 or mouse erythrocyte receptors. The malignant counterparts of the mature B cell are the follicular small cleaved and large cell lymphomas and the diffuse small cleaved and large cell lymphomas and PL cells. At the next step of maturation, the plasmacytoid B cell secretes Ig, usually of the IgM subclass, and expresses new surface membrane antigens including OKT10, PCA-1, and PC-1. It has recently been demonstrated that hairy cell leukemia falls somewhere between the mature B cell and plasma B cell; they also express the PCA-1 antigen.[302] The plasma cell, the most differentiated B lymphocyte, expresses the same phenotype as myeloma cells. Although these cells have CIg, produce immunoglobulin, and express OKT10, PC-1, and PCA-1, they lose other surface membrane markers, including SmIg, Ia, and B cell antigens.

T cell differentiation follows a distinct pathway. Early thymocytes (stage I) express Leu-9, Leu-1, T9, T10, and often T11/Leu-5 (sheep erythrocyte receptor); this phenotype probably represents the malignant counterpart of group I T-ALL cells. A case has been made that the stage I thymocyte has not yet rearranged the $T\beta$ receptor gene[212]; this remains to be confirmed. The common thymocyte (stage II) no longer expresses T9; it gains T6 antigen and simultaneously expresses the helper-associated (T4/Leu-3) and suppressor-associated antigens (T8/Leu-2). This cell clearly rearranges the $T\beta$ gene, confirming that $T\beta$ rearrangement precedes surface membrane expression of the T3–Ti complex.[212] This cell corresponds to the phenotype of group II T-ALL and some lymphoblastic lymphomas. Subsequently, the cells lose either the helper-associated or suppressor-associated antigens. This is equivalent to group III T-ALL or some cases of lymphoblastic lymphoma. In the final stage of maturation, the suppressor-associated cell (T8/Leu-2) may express the receptor for the Fc portion of IgG as well as the surface markers previously attributed to the mature thymocyte (including the T3–Ti complex), coinciding with the phenotype of some T-CLL cells and chronic $T\gamma$ lymphoproliferative disease. The helper-associated mature T lymphocyte (T4/Leu-3), on the other hand, may express the receptor for the Fc portion of IgM and coincides with the phenotypes of some T-CLL, adult T cell leukemia/lymphoma, cutaneous T cell lymphoma, and peripheral T cell lymphoma.

CLASSIFICATION OF THE NONLYMPHOID LEUKEMIAS AND LYMPHOMAS

Hodgkin's disease. Hodgkin's disease (HD) is a malignant neoplasm of uncertain cellular origin characterized by the appearance of distinctive binucleate or multinucleate giant cells (Reed-Sternberg cells, RSCs) and their mononuclear variants (Hodgkin's cells, HCs).[311] The malignant nature of this disease is suggested by cytogenetic studies that have shown a clonal distribution of chromosomal aneuploidy.[312–314] Considerable debate has arisen as to what constitutes the malignant cell of HD. However, most investigators now agree that the RSCs or HCs (a subset constituting a minute fraction of the tumor mass) represent the neoplastic cell population.[315] The normal cellular counterpart from which RSCs and HCs arise has not yet been identified.[315]

Investigators have used morphology (light and electron microscopy), cell culture, and immunohistochemistry in an attempt to characterize the nature of the RSCs and HCs. Based on these observations, it has been argued that HD arises from the T lymphoid,[316,317] B lymphoid,[318–328] or myeloid-macrophage lineages.[311,329–331] Although this controversy is unresolved, the application of immunologic marker analysis has contributed to our further understanding of the disease, and several general statements can be made. First, with few exceptions, most observers have failed to detect the uniform expression of T cell surface markers (as defined by polyclonal and monoclonal reagents)[328,332–337] by RSCs or HCs, suggesting that these cells are not of T lymphocyte origin. RSCs and HCs have been shown to express the Tac antigen (IL-2 receptor)[338]; in two reports, these cells were found to be T9 positive (transferrin receptor).[332,334] Neither receptor-associated marker is restricted to the T cell lineage.[304,339] Second, although the detection of SmIg or CIg in RSCs and HCs favors a B cell origin,[318,319,322] the expression of these determinants is often polyclonal,[321,340–344] which suggests that immunoglobulin is adsorbed onto RSC and HC cells rather than being synthesized by the malignant cell.[340] There are no convincing data that RSCs or HCs produce immunoglobulin. Immunologic staining of RSCs and HCs for the expression of B cell differentiation antigens has produced conflicting results.[332,333,345] In an interesting case of B cell HD,[346] a patient with nodular sclerosing HD developed a terminal leukemic phase. The circulating HCs expressed the B1 and B4 antigens and had cytoplasmic μ heavy chains and a clonal rearrangement of heavy and light chains (consistent with a B cell origin). Substantial data, however, favor a myeloid-macrophage origin for HD.[340,341,344,347–349] This conclusion is based on the demonstration of nonspecific esterase (NSE) and acid phosphatase, α-1-antitrypsin and α-1-antichymotrypsin, muramidase, lectin-binding properties, and the variable expression of Fc and C3 receptors on

RSC and HC cells. Although short-term cell lines believed to be derived from RSCs demonstrate weak phagocytic activity, and one line was reported to synthesize IL-1,[347,349] other established cell lines have not uniformly shown these activities.[350] In most instances, RSCs and HCs do not react with antibodies to monocytes,[318,332-336,345,350] although in one report a substantial number of biopsy specimens contained RSC and HC positive for markers characteristic of late granulocytic maturation (TU5, TU6, TU9).[333,351] Based on Ia expression[318,332,336,345] and characteristic cytochemical features, other authors have suggested that the cell of origin for HD is a "reticulum cell" (either a dendritic cell or an interdigitating reticulum cell).[336,352] Finally, some data suggest that the RSCs and HCs represent a subset of activated lymphoid cells of either T or B lymphoid origin. This conclusion is based on an immunologic analysis in which RSC and HC uniformly expressed the Ki-1 marker (35 of 35 biopsy specimens of all histological subtypes) as defined by a monoclonal antibody raised by immunization against an established HD cell line.[353] Among normal cells, Ki-1 is expressed by T and B lymphocytes activated in vitro by various stimuli that also induce interleukin-2 (IL-2) receptor expression.[337] In situ staining of biopsy specimens from nonneoplastic and reactive tissues demonstrated Ki-1 expression by a population of normal perifollicular lymphoid cells (lymph node and spleen) and variable degrees of expression by abnormal lymphoid cells in cases of angioimmunoblastic lymphadenopathy and lymphatoid papulosis. Among 290 cases of non-Hodgkin's lymphoma, Ki-1 expression was observed in 19 cases of peripheral T cell lymphoma and in 45 cases of diffuse large cell lymphoma (including 35 specimens expressing T cell surface markers and 7 bearing B cell antigens). These results suggest that Ki-1 is a lymphoid activation antigen that identifies a group of large lymphoid cells in normal and neoplastic tissues (including RSCs) that remains poorly characterized. Another monoclonal reagent, HeFi-1, is similar if not identical to Ki-1.[354]

In summary, the cellular origin for HD remains unclear. Although Ia and T9 antigen staining of RSC and HC cells have been reported, Ki-1 antigen expression may prove to be the most useful immunologic marker for this disease.

RSCs and HCs constitute only a small portion of cells within the tissue of Hodgkin's disease. Recent efforts are directed toward characterizing the remaining cells and have been recently reviewed.[355] Use of in situ techniques has demonstrated that lymphoid tissues involved with HD appear to be heterogeneous in immunohistologic make-up; some cases demonstrate numerous T lymphocytes with few B cells, whereas others exhibit prominent follicles of polyclonal B lymphocytes and only small numbers of T cells within these follicles. In two studies,[335,355] these B cell-rich cases were of the lymphocyte-predominant type. In specimens containing T lymphocytes, RSCs and HCs tend to appear in areas of heaviest T cell infiltration, suggesting a relationship. Many cells within areas of T cell infiltration are Ia or T10 positive, suggesting that these T lymphocytes are activated. Several investigators have demonstrated that most HD-associated T lymphocytes are of the helper cell subset.[332,335,355-357] Genotyping for immunoglobulin and T cell receptor rearrangements may lead to a better understanding of the cellular origin of this disease.

Malignant disorders of macrophages. Several malignant diseases of macrophages (or histiocytes) have been described.[358,359] The term histiocytic lymphoma, used in the Rappaport classification,[215] encompasses a heterogeneous group of neoplasms of large transformed lymphocytes and, rarely, of macrophages.[360,361]

The malignancies of macrophages (histiocytosis X) are heterogeneous.[362] Clinical presentations include solitary benign eosinophilic granuloma, Hand-Schuller-Christian disease, and histiocytic medullary reticulosis; the latter is a generalized systemic disorder characterized by fever, wasting, hepatosplenomegaly, variable lymphadenopathy, and progressive pancytopena due to diffuse tissue invasion by malignant macrophages.[363] The equivalent disease in children is sometimes referred to as Letterer-Siwe disease. Because of morphologic and ultrastructural similarities between malignant macrophages and epidermal Langerhans cells, it has been proposed that these diseases represent a proliferative disorder of Langerhans cells.[364-366] Both cell types possess receptors for C3 and the Fc portion of IgG[367] and express the T6 and Ia antigens.[368,369] The malignant macrophages also express the OKM1 and other macrophage surface markers.[370] An unexpected and unexplained finding was the presence of the T4 antigen in this disorder.[368]

AML. AML is a clonal malignancy of myeloid progenitor cells resulting in excessive proliferation and accumulation of immature hematopoietic elements. The subtypes of this disease are generally classified according to the morphologic similarity of the leukemic cell population to normal myeloid precursors, eg, acute myeloblastic leukemia, acute promyelocytic leukemia, acute monoblastic leukemia, and acute erythrocytic leukemia.

Monoclonal antibodies have been evaluated for reactivity against AML cells (Tables 9 and 10). In all cases, these antibodies identify determinants expressed by either normal circulating myeloid cells or bone marrow progenitors. None of these reagents recognizes a leukemia-specific determinant and, with few possible exceptions,[371-373] attempts to generate leukemia-specific antisera have been unsuccessful. Studies of the reactivity of antimyeloid cell antibodies for AML cells have raised several issues: (a) whether these reagents are specific in their reactivity for myeloid *v* lymphoid leukemia cells; (b) whether antibody reactivity correlates with classification using the FAB nomenclature; (c) whether surface marker expression by myeloid leukemia cells corresponds to stages of normal myeloid differentiation and, if so, whether this is of prognostic significance; (d) whether myeloid leukemia cells in a patient are homogeneous in their expression of surface markers; (e) whether leukemia progenitor cells defined by their ability to form leukemic colonies in vitro exhibit the same antigenic phenotype as their progeny in bone marrow and blood; and (f) whether monoclonal antibodies that identify antigens expressed by myeloid leukemia cells can be used for immunotherapy.

AML v the lymphoid leukemias. Because of differences in prognosis and therapy, it is important to distinguish between AML and the lymphoid leukemias. Although differ-

Table 9. Use of Myeloid Surface Markers to Discriminate Between Acute Myeloid and Lymphoid Leukemia

Monoclonal Antibody	Myeloid Leukemia			Lymphoid Leukemia			References
	AML	CML (MBC)	Total Myeloid	ALL	CML (LBC)	Total Lymphoid	
Mo1/OKM1	63 (228)*	57 (23)	62 (251)	0 (82)	0 (11)	0 (92)	66,106,118,124,389,419
MY7	76 (97)	92 (13)	78 (110)	1 (109)	0 (13)	1 (122)	118,119,419
MY8	53 (73)	7 (15)	45 (88)	0 (82)	0 (11)	0 (83)	118,119,419
MY9	85 (97)	92 (13)	85 (110)	2 (109)	0 (13)	2 (122)	70
VIM-2	91 (66)	93 (30)	92 (96)	5 (60)	0 (11)	4 (71)	106
VIM-D5	68 (116)	88 (8)	69 (174)	2 (88)	NR	2(88)	389,418

MBC, myeloid blast crisis; LBC, lymphoid blast crisis.

*Percentage of patients positive (total number of patients tested). A patient is considered positive for a given marker if >20% of malignant cells bind the monoclonal antibody.

ences in morphology and histochemistry often lead to the correct diagnosis, the distinction between immature variants of AML and ALL is not always evident. Monoclonal reagents that identify antigens expressed by myeloid but not lymphoid leukemias (or vice versa) would therefore be important. Six monoclonal antibodies have been extensively tested for reactivity to myeloid and lymphoid leukemia (Table 9). Each of the six antigenic determinants defined by these antibodies (Mol/OKM1, MY7, MY8, MY9, VIM-2, and VIM-D5) is expressed by more than one half of patients with AML (53% to 91%), defined as antibody binding by >10% to 20% of malignant cells in each patient. The myeloblasts of patients with myeloid blast crisis of CML demonstrate similar frequencies of expression for these determinants except for MY8. Conversely, expression of these antigens on acute lymphoid leukemia cells (including the T and B cell variants of ALL and chronic myelogenous leukemia (CML) lymphoid blast crisis) is rare. Clearly, these monoclonal reagents can complement other tests in the differential diagnosis of AML versus ALL. The accuracy of immunologic diagnosis can be extended by using more than one antimyeloid reagent in conjunction with antibodies that detect antigenic determinants uniquely expressed by B or T lymphoid leukemias (anti-CALLA, B1, B4, OKT3, Leu-4, etc.) (see above). Occasionally, however, this approach has produced seemingly disparate results, with the detection of leukemia cells with myeloid and lymphoid differentiation markers.[374–376] These rare situations may reflect the existence of biopotential clones of malignant cells expressing features of more than one lineage.[377]

Correlation between surface marker phenotype and FAB classification. There are several types of AML differ in morphology, histochemistry, and surface marker expression. Classifications have been proposed to identify these types, based on the hypothesis that this information may be of prognostic and therapeutic significance. The FAB group classification, which relates the morphologic appearance of leukemic cells to presumed normal hematopoietic counterparts, is widely used.[185,186,378,379] Seven subtypes of AML (M1 through M7) are identified: M1 and M2 represent undiffer-

Table 10. Correlation Between Myeloid Surface Marker Expression and FAB Classification System

Monoclonal Antibody		FAB Classification					References
		M1	M2	M3	M4	M5	
VIM-2	(M + N)*	73 (15)†	96 (23)	83 (6)	100 (16)	100 (6)	106
R1B19	(N)	40 (10)	44 (9)	13 (8)	48 (27)	38 (8)	67,68
S4-7	(M + N)	50 (10)	56 (9)	25 (8)	70 (27)	78 (9)	67,68
PM 81	(M + N)	90 (10)	50 (2)		86 (7)	100 (3)	97,122
MY9	(M)	85 (54)‡		100 (6)	81 (31)	83 (6)	70
MY7	(M + N)	78 (54)		67 (6)	81 (31)	50 (6)	118,119
Mo5	(M + N)	50 (38)			74 (27)‡		95
MOP9	(M)	17 (6)	13 (8)	0 (3)	100 (7)	100 (10)	389
AML-2-23	(M + N)	0 (12)	0 (2)		78 (9)	100 (5)	121,122
MY4	(M)	25 (36)		0 (3)	52 (25)	100 (6)	119
UCHM1	(M)	6 (17)			92 (24)	100 (16)	66
MY8	(M + N)	36 (39)		33 (3)	76 (25)	83 (6)	118,119
Mo1/OKM1	(M + N)	39 (124)		100 (3)	91 (101)		66,106,118,124,389
Mo2	(M)	14 (65)			45 (31)		124
VIM-D5	(N)	29 (38)	71 (52)	67 (12)	88 (40)	92 (24)	389,418
82H5	(N)	0 (5)	69 (13)	100 (4)	100 (6)	100 (7)	83

*Antigen expression by peripheral blood monocytes (M) or neutrophils (N).

†Percentage of patients positive (total number of patients tested).

‡Patients with M1 and M2 or M4 and M5 leukemia combined.

entiated and differentiated myeloblastic leukemia; M3, promyelocytic leukemia; M4 and M5, myelomonocytic and monocytic variants; M6, erythroleukemia; and M7, megakaryocytic leukemia. Although some investigators have reported briefer remissions or lower response and survival rates in patients with the M5 variant,[380-382] fewer remissions in erythroleukemia (M6),[380] or longer remissions in promyelocytic leukemia (M3),[380-382] these observations are controversial.[186,381-383] With the development of immunologic reagents that detect antigenic markers expressed by normal and leukemic myeloid cells, analyses have been undertaken to compare the FAB system with patterns of surface marker expression. Table 10 indicates 16 monoclonal reagents whose relative reactivity against FAB-classified AML variants can be critically assessed. Within the first group of seven antibodies (VIM-2, R1B19, S4-7, PM81, MY9, MY7, and Mo5), no clear distinction exists in antigen expression by cells in each of the five FAB variants (M1-M5; too few patients with the M6 and M7 variants were examined to draw conclusions). The frequency of expression by patients in each subclass is generally 50%. In the second group of seven antibodies (MOP9, AML-2-23, MY4, UCHM1, MY8, Mo1/OKM1, and Mo2), there is a trend toward higher frequency of antigen expression among individuals whose leukemia cells display monocytic differentiation (M4 + M5). In the case of VIM-D5 and 82H5, only undifferentiated M1 cells have a lower frequency of expression. None of these reagents demonstrates preferential binding frequency to M1 and/or M2. One antibody, VIE-64, which binds to glycophorin A, displays relative binding specificity toward M6 variant cells.[384] Monoclonal antibody SFL 23.6 has a well-defined reactivity restricted to the erythroid lineage including erythroleukemia cell lines and should be useful in distinguishing M6.[125] Monoclonal antibodies against platelet glycoproteins Ib, IIb/IIIa, and aIIIaa, for factor VIII-related antigen can be used to identify megakaryoblasts.[378] With these possible exceptions, the degree of correlation between surface marker expression and the criteria for FAB classification is not convincing.[385,386]

Subclassification of AML according to differentiation-associated phenotypes as identified by monoclonal antibodies. Because there is controversy over whether the FAB classification system provides prognostic information, alternative classifications have been proposed. In a surface marker analysis of 70 patients with AML, Griffin and co-workers[119] identified four phenotypes based on patterns of surface antigen expression that correlated with phenotypes displayed by myeloid cells during normal differentiation. Group I AML cells (21% of patients) expressed the antigenic phenotype of the CFU-C–committed myeloid progenitor cell (Ia and MY7-positive); group II cells (26%) displayed the phenotypic characteristic of normal myeloblasts (MY7, Ia, and Mo1/OKM1 and My8-positive); group III cells (8%) had a phenotype featured by normal promyelocytes (MY7 and Mo1/MY8-positive; Ia-negative); and group IV cells (45%) with the phenotype of promonocytes and monocytes (MY4, MY7, MY8/Mo1, and Ia-positive). Within these four differentiation-related groups there was considerable morphologic heterogeneity: although all three of the promyelocytic leukemia (M3) patients were in group III and all six monocytic leukemia (M5) patients were in group IV, the myeloid leukemia (M1 and M2) and myelomonocytic leukemia (M4) patients were dispersed throughout all four groups, with a tendency for myeloid patients to be in groups I, II, and III, and myelomonocytic patients to be in groups II and IV. The preliminary finding of a larger prospective analysis involving over 200 patients demonstrates significant differences among these phenotypic groups with respect to complete response rate and disease-free survival (J.D. Griffin, personal communication). Moreover, expression of certain markers appears to be of independent prognostic significance: AML patients with MY7-positive leukemia exhibit a worse prognosis than do MY7-negative patients; the expression of monocyte antigen MY4 is also predictive of a poor response. Several studies of the biological implications of surface marker phenotype in AML are in progress.

A scheme for myeloid differentiation is shown in Fig 1. In an attempt to account for the FAB M4 leukemic cell (bearing features of both granulocytic and monocytic differentiation), Ball and Fanger[122] have proposed that the normal M4 counterpart is an intermediate bipotential precursor cell capable of differentiating along either the monocytic or granulocytic path of differentiation. They further suggest that the myeloblast (M1 and M2), the progenitor of the M4 cell, is likewise bipotential. Given the ability of the promyelocytic leukemia cell line HL-60 to undergo subsequent differentiation toward mature monocytes or neutrophils depending on the nature of the inducing stimulus,[387,388] it appears that the normal promyelocyte is not irreversibly committed to granulocytic maturation. Although these hypotheses are consistent with some experimental observations, considerable additional data are required.

Heterogeneity of surface marker expression by malignant AML cells. Most studies of AML indicate considerable heterogeneity in leukemic cell surface marker expression between patients as well as within a given individual.[62,67,69,95,114,118,122,386,389] Typically, a patient is classified as positive for the expression of a marker if >10% to 20% of the patient's leukemia cells display the determinant. Although certain antigens tend to be expressed by >50% of the leukemic cells of given individuals, variability is considerable. If surface marker expression correlates with the level of myeloid differentiation, these data suggest that the leukemic population is heterogeneous.

AML likely arises from leukemic myeloid progenitor cells, which in some cases can be grown in vitro in semisolid medium.[390-392] The clonogenic leukemia cells (L-CFC) are, by definition, capable of limited proliferation (with a subset capable of self-renewal), a feature that distinguishes them from most leukemia cells that are terminally differentiated.[392,393] Several groups have recently investigated the surface marker characteristics of L-CFC and compared them with the total leukemia population.[70,114,394,395] The surface-marker phenotype of the total leukemia population, as determined by immunofluorescence analysis, may not predict the phenotype of the L-CFC as measured by inhibition of L-CFC growth after antibody-dependent, complement-mediated lysis. In general, the L-CFC has a pattern of

antigenic expression that is more "immature" than that of the predominant phenotype of the total population. L-CFC have been subclassified using multiple markers whose expression on normal CFU-GEMM (Ia, MY9, S3-13, S8-6), early (day 14) CFU-GM (Ia, MY9, PM-81, S3-13, S8-6, S4-7), and late (day 7) CFU-GM (all of the preceding markers plus AML-2-23 and R1B19) are known. Three phenotypically distinguishable levels of differentiation have been identified.[114,394] The degree of maturity, as based on morphology (FAB classification) and expression of "later stage" antigens (beyond the CFU-GM: Mo1, MY3, Mo2) of the total leukemia population, tends to correlate with the L-CFC maturation level (eg, CFU-GEMM level L-CFC are associated with M1 morphology and lack expression of late antigens), suggesting a limited potential for terminal differentiation[114,395] These data suggest that L-CFC are a distinct subset of clonogenic cells among the total leukemia population; these cells may arise at multiple points along the pathway of early myeloid differentiation.

CML. CML is a myeloproliferative disorder characterized by a consistent chromosomal abnormality, the Philadelphia (Ph^1) chromosome. The Ph^1 chromosome results from a reciprocal translocation between chromosomes 9 and 22 designated t(9;22).[396] This translocation results in the transfer of the c-*abl* oncogene from chromosome 9 to the Ph^1 chromosome and the variable reciprocal translocation of c-*sis* from chromosome 22 to 9.[397] The target for leukemic transformation (Fig 3) appears to be at the level of the pluripotential stem cell, since the Ph^1 chromosome is present in all hematopoietic elements of patients with CML, including B and T cells. [398–404] The clonal origin of CML is further indicated by analysis of patterns of expression of glucose-6-phosphate dehydrogenase (G6PD), and adenylate kinase isoenzyme.[398,402] In the chronic phase of the disease, CML is characterized by an overproduction of relatively mature granulocytes. After a variable period of time, with a median of 3 years, most patients enter an acute phase (blast crisis) in which maturation no longer occurs. The acute phase resembles acute leukemia. Approximately one-third of patients with acute-phase CML demonstrate cells with lymphoid features that include the expression of TdT, CALLA, Ia, B1, rarely Cμ and rearrangements in immunoglobulin heavy and light chain genes.[403,405–411] Rare cases of lymphoid acute phase with T cell markers have also been reported.[412–414] Acute phase CML involving myeloid cells is heterogeneous; typically the cells resemble myeloblasts, but erythroblasts, megakaryoblasts, and monoblasts can also be observed. Distinction between lymphoid and myeloid acute phase is important because patients with lymphoid blast crisis may respond to chemotherapy with vincristine (V) and prednisone (P).[410,415–417] In making this diagnostic distinction, the characteristic expression of several myeloid markers (MY7, MY9, VIM-2, and Mol/OKM1) on myeloid blast crisis cells and their lack of expression by lymphoid blast crisis cells (Table 9) provide information complementary to assays for the detection of CALLA, B1, TdT, and CIg.[38,66,70,105,106,118,119,124,409,418,419]

Surface marker analysis may allow further discrimination among the heterogeneous presentation of CML acute phase.

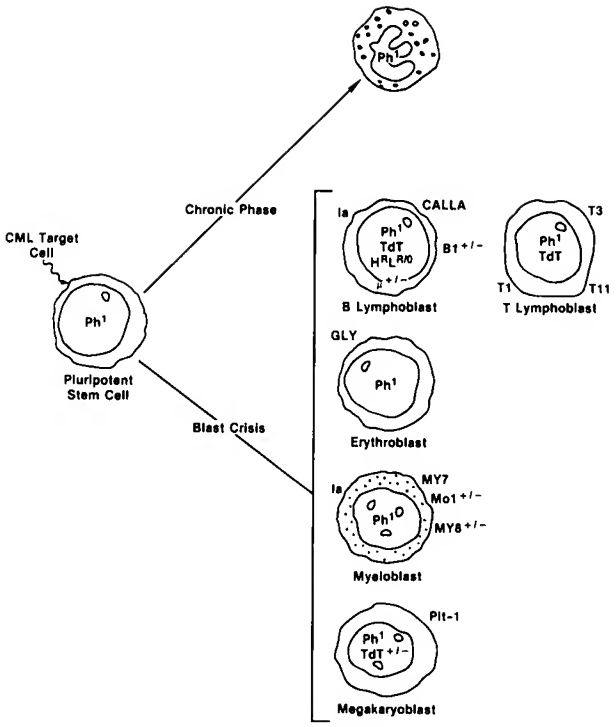

Fig 3. Schematic representation of the origin of chronic and blast crisis phase of chronic myelogenous leukemia (CML) from the target pluripotent stem cell. Phenotypes for various forms of CML blast crisis (based on data of Griffin et al[418]) are indicated. Ph^1, Philadelphia chromosome; TdT, terminal deoxynucleotidyl transferase; GLY, glycophorin A.

Four phenotypes were identified in 30 patients with this disorder based on antigen expression of normal myeloid, erythroid, megakaryocytic, and lymphoid cells.[419] The cells of ten patients exhibited a phenotype corresponding to an immature myeloid cell (Ia, MY7, and Mol-positive); all ten were negative for CALLA, B1, and TdT. These cases were felt to represent "myeloid" blast crisis; none of these patients responded to treatment with V and P. Cells from 11 patients expressed a phenotype similar to acute (early B) lymphoblastic leukemia cells (TdT, Ia, CALLA, and B1-positive; MY7 and Mol-negative); six of nine evaluable patients had a complete response to V and P. Cells from one patient had the phenotype of erythroleukemia (glycophorin A-positive); another patient's cells expressed the phenotype of megakaryocytic leukemia (Plt-1-positive); one patient's cells had features of both myeloid and lymphoid blasts on different cells. Cells from six patients did not express surface markers characteristic of any lineage; these were termed "undifferentiated"; these cases were heterogeneous in expression of TdT; no complete responses to V and P were observed. Thus, in terms of response to V and P therapy, surface marker analysis provided useful prognostic information.

In another series of 45 patients with CML in blast crisis, 28 patients were classified as having "myeloid" blast crisis on the basis of reactivity with at least one of six antimyeloid monoclonal reagents (including VIM-D5 and VIM-2) and no surface expression of T or B cell immune markers or

TdT.[420] Among these myeloid cases, however, only 11 patients expressed granulomonocytic antigens exclusively; the blast cells of 17 patients were additionally positive for platelet/megakaryocyte markers (16 cases) and/or erythroid determinants (three cases). Whether the *same* blast cell co-expressed myeloid, megakaryocytic, or erythroid antigens was not determined. Fourteen patients were classified as having "lymphoid" blast crisis with the phenotypic pattern of CALLA-positive ALL (10 cases), pre-B cell ALL (three cases), or "Null" ALL (one case). Two patients demonstrated a mixed myeloid and lymphoid blast cell phenotype, and a single patient was unclassifiable. Sixteeen of these 45 patients were tested serially during the course of their illness and three demonstrated phenotypic changes. The immunological diagnosis of lymphoid blast crisis was associated with a higher rate of remission than was myeloid blast crisis (57% *v* 4%, respectively) and a longer median survival.

Certain patients with the Ph^1-chromosome are first diagnosed in the acute phase without a preceding history of a chronic phase.[415] Either a chronic phase never existed or it was never detected. Surface-marker analysis may be as useful in the subclassification of these patients' cells as it is in the more typical acute phase that is preceded by a chronic phase.

MONOCLONAL ANTIBODY THERAPY

Several investigators[421-431] have attempted to treat lymphoid or myeloid leukemias with monoclonal antibodies. In some studies, patients with advanced B cell-derived CLL received T101 monoclonal antibody.[421,422] T101 could be safely infused and led to transient reductions in circulating leukemia cells; there was, however, no sustained effect on the bone marrow, involved lymph nodes, or other organs. This therapy resulted in some intravascular cell injury, but destruction in the spleen, liver, and lungs was probably more important. Similar results have been reported in patients with adult T cell leukemia/lymphoma, ALL, and AML treated with other monoclonal antibodies.[423-425] Patients with cutaneous T cell lymphoma who received T101 or anti-Leu-1 have had only transient improvement in skin lesions and lymphadenopathy.[426-429] Side effects of monoclonal antibody therapy are usually minor. Respiratory distress following the rapid infusion of monoclonal antibody has been described,[422] and some patients have demonstrated transient elevation of serum creatinine and hepatic enzymes.[423]

Monoclonal antibody therapy has several shortcomings that must be addressed. First, treatment with antibodies such as T101 results in modulation of the antigen from the cell surface, which prevents antibody binding to the tumor cells. The T101 antigen-antibody complex is pinocytosed into the cytoplasm,[430] a phenomenon that might be advantageous when drugs or toxins are linked to the antibody to enhance its cytotoxicity. Antigen in the circulation poses another potential problem because it might prevent the antibody from reaching the tumor cells. Furthermore, murine antibodies can stimulate production of human anti-mouse antibodies which lead to antibody neutralization. This situation may be correctable by treatment with high initial doses of antibody (> 500 mg) or by simultaneous treatment with immunosuppressive drugs to induce tolerance. In addition, the heterogeneity of antigen expression of tumor cells may necessitate therapy with more than one antibody. Clearly, monoclonal antibody therapy for leukemia and lymphoma is in its earliest stages.

An interesting therapeutic approach with monoclonal antibodies involves the use of anti-idiotype monoclonal antibody reactive with the idiotype of the immunoglobulin on malignant B cells. Such an antibody is by definition specific for a patient's tumor cells. A patient with B cell lymphoma in an accelerated phase who was unresponsive to conventional therapies was treated with an IgG_{2b} anti-idiotype monoclonal antibody.[431] Following eight intravenous (i.v.) infusions, the patient entered a complete remission that has been sustained for >3 years. Results were less impressive in other lymphoma patients treated with this approach with ~50% achieving short-lived partial remissions.[432,433] We developed several monoclonal anti-idiotype antibodies to cells from patients with leukemia and lymphoma.[268] The first patient to undergo treatment had advanced CLL. Sequential anti-idiotype monoclonal antibody therapy with IgG_{2b} and IgG_1 antibody provided no benefit. His therapy was limited because of circulating idiotype immunoglobulin that blocked the binding of the anti-idiotype antibody to the leukemia cells. We were able to reduce the circulating idiotype sufficiently with extensive plasmapheresis.

Although anti-idiotype antibody therapy remains an interesting area of investigation, its applicability is limited by patient specificity (ie, antibodies are "tailor-made" for a single patient) and the presence of antibody in the serum of many patients. Recent data indicate that some tumors are biclonal; this would require the use of more than one antibody.[434,435] In addition, the tumor cell idiotype may be unstable due to somatic mutation within the immunoglobulin variable region genes.[436,437]

A number of centers are studying toxin and drug conjugates with murine antibodies directed toward human tumors; clinical trials have just begun. Antisera and monoclonal antibodies conjugated to radionuclides for tumor imaging have been extensively studied; this subject was recently reviewed.[438] We have used the T101 antibody conjugated to ^{111}indium for imaging in 12 patients with cutaneous T cell lymphoma.[439,440] Tumors as small as 0.5 cm have been localized; however, nonspecific uptake of the immunoconjugate in the liver and spleen has prevented critical evaluation of these organs. This difficulty has been partially circumvented by the administration of intracutaneous injections of the immunoconjugate which cause it to be carried via the lymphatics directly to lymph node sites of disease.[441] This procedure does not, of course, facilitate visualization of extralymphatic disease.

Survival for patients with ALL following relapse has not improved over the past several years with chemotherapy drugs. Allogeneic bone marrow transplantation clearly leads to improved survival, but only 30% to 40% of patients have matched donors.[442-444] An alternative method to allogeneic bone marrow transplantation would make use of monoclonal antibodies to cleanse autologous bone marrow prior to bone marrow transplantation. Patients who are in clinical remis-

sion are likely to have morphologically undetectable tumor cells in their bone marrow; these cells may be identified and destroyed in vitro by specific antibodies and complement or antibodies conjugated to toxins. In one recently reported study, patients with ALL in second or subsequent remission had their bone marrow treated with a mixture of the BA-1, BA-2, and BA-3 monoclonal antibodies and rabbit complement.[445] All the patients were prepared for transplantation with cyclophosphamide and fractionated total body irradiation. Engraftment occurred in all the patients and 7 of the 23 patients were relapse-free from 6 to 32 months (median 21 months) posttransplantation. All but one of the deaths was caused by recurrent leukemia. The researchers concluded that autologous bone marrow transplantation using in vitro-treated marrow was safe, allowed engraftment, and resulted in prolonged survival in some patients with ALL in second or subsequent remission. Similar results have been reported for ALL patients treated with the J5 monoclonal antibody and complement.[446] Relapse of leukemia in these patients may result from the inadequacy of the preparative regimen used to treat the patients prior to transplantation, inadequate removal by the in vitro treatment with monoclonal antibody and complement, or possibly to the lack of the putative graft *v* leukemia effect described in allogeneic bone marrow transplantation.[447] Even in allogeneic transplantation, in which the preparative regimens are identical to those of autologous transplantation, >50% of the ALL patients relapse, suggesting that an insufficient preparative regimen may be the factor leading to relapse in autologous transplantation as well.

In another study, patients with advanced B cell non-Hodgkin's lymphoma underwent in vitro bone marrow treatment with the anti-B1 antibody and complement.[448] Ten of 17 patients are disease-free at a median follow-up of 22 months (L.M. Nadler, personal communication). Despite the presence of the B1 antigen on mature B cells, B cells recovered within the first few months after transplantation, suggesting that the normal B cell progenitor does not express the B1 antigen.

Another approach to cleansing bone marrow in vitro is the use of monoclonal antibodies conjugated to toxins. In one study, whole ricin was conjugated to the T101 and 3A1 antibodies.[449] It was demonstrated that 95% of the tumor colonies were killed whereas 96% of bone marrow progenitor cells survived. Similar results were reported for a panel of anti-T cell monoclonal antibodies conjugated to intact ricin.[450] Other investigators reported results of an immunotoxin synthesized with pokeweed antiviral protein and the B43 antibody directed against Burkitt lymphoma cells.[451] Immunotoxins may prove to have advantages over antibody and complement; not all antibodies fix complement, and immunotoxins may have greater cytotoxic capability.

The use of monoclonal antibodies and antibody immunoconjugates in the treatment and radioimaging of cancer is in its infancy. Although much work must still be done to address the problems of monoclonal antibody therapies, studies in animal tumor models and humans have clearly demonstrated that antibodies alone or antibody conjugates can be safely administered with minimal adverse effects; in selected cases, these may have diagnostic and therapeutic value. Nonspecific localization of antibody in the reticuloendothelial system, host antibody response, and antigenic heterogeneity are major obstacles to safe and effective treatment with monoclonal antibodies. These issues are under investigation in animal models and humans. Although anti-idiotype antibodies are highly specific and have produced excellent responses in a small number of patients, problems such as biclonality of some lymphomas, instability of the idiotype, and the difficulty of tailoring antibodies to individual patients clearly limit the role of anti-idiotype therapy. The utility of purging bone marrow in vitro with antibodies and complement (or antibodies coupled to toxins) is limited to only a few diseases. However, studies have demonstrated that tumor cells can be removed from the bone marrow following in vitro treatment with antibody and complement; treated bone marrow can successfully engraft, and a number of patients have remained disease-free for >2 years. Whether this is related to the in vitro treatment is unknown. This treatment may prove to be an important application of monoclonal antibody therapy, and it bypasses most of the problems associated with in vivo monoclonal antibody serotherapy. Perhaps the most important future role for monoclonal antibody therapy will be in patients with minimal disease in the "adjuvant" setting, in whom antibody conjugates may eliminate micrometastatic deposits of tumor cells. This remains to be addressed in controlled trials.

CONCLUSION

The application of hybridoma technology and the exciting discoveries in molecular biology over the past 10 years have led to major advances in our understanding of the cellular origin of leukemia and lymphoma and will likely lead to a better understanding of the etiology of these diseases. Utilizing these techniques, it is now possible to more accurately diagnose and classify these disorders, sometimes guiding therapeutic decisions. It is also possible to use molecular probes to detect minimal residual disease. In the future, monoclonal antibodies conjugated to isotopes, drugs, and/or toxins will likely have a role in the therapy of certain leukemias and lymphomas. We look forward to this exciting new era in cancer therapy and diagnosis.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Goldenberg

Serial No.: 10/002,211

Filed: December 5, 2001

Title: METHOD OF TREATING IMMUNE DISEASE USING B-CELL ANTIBODIES

Group Art Unit: 1644

Examiner: Chun Crowder

Attorney Docket No.: IMMU:003US1

EFS-WEB

DECLARATION UNDER 37 CFR §1.132

MAIL STOP AMENDMENT
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Sir:

I, Thomas Dörner, being duly warned, declare as follows:

1. I am a Professor of Medicine in the Department of Medicine/Rheumatology and Clinical Immunology, Charite Hospital, Berlin, Germany. I have an extensive background in autoimmune diseases and in the field of immunotherapy for the treatment of autoimmune diseases, as evidenced by my Curriculum Vitae, which is attached. In particular, I have been the principal investigator on clinical trials relating to immunotherapy of various autoimmune diseases with B-cell antibodies and TNF inhibitors. I have been the principal investigator of a study of the use of Immunomedics' antibody epratuzumab in systemic lupus erythematosus, for which Charite Hospital received grant funds. Dr. Goldenberg and I were co-authors on a paper publishing the results of this study ("Initial clinical trial of epratuzumab (humanized anti-CD22 antibody) for immunotherapy of systemic lupus erythematosus" *Arthritis Res Ther.* 2006;8(3):R74. Epub 2006 Apr 21), and Immunomedics covered a portion of my traveling expenses for presenting this paper at a scientific meeting. I have co-authored two other papers with Dr. Goldenberg (*Arthritis Rheum.* 2006 Jul;54(7):2344 and *Ann Rheum Dis.* 2007 Aug 2; [Epub ahead of print]). I have known Dr. Goldenberg of Immunomedics professionally for several years as a researcher in the field, and we interact at meetings and when we discuss

science. I have interacted with him twice when we attended scientific meetings in the past 3 years. I am being compensated on an hourly basis for my time in connection with this declaration.

2. I have read the Official Action dated July 26, 2007, for the above-captioned case. I have also reviewed the currently pending claims for this case and read the specification. I note in particular the following disclosures in the specification:

- "ablation of certain normal organs and tissues for other therapeutic purposes, such as the spleen in patients with immune disease or lymphomas, the bone marrow in patients requiring bone marrow transplantation, or normal cell types involved in pathological processes, such as certain T-lymphocytes in particular immune diseases" (page 7, lines 5-10)

- Another therapeutic application for such organ- and tissue-targeting antibodies conjugated with a toxic agent is for the ablation of certain normal cells and tissues as part of another therapeutic strategy, such as in bone marrow ablation with antibodies against bone marrow cells of particular stages of development and differentiation, and in the cytotoxic ablation of the spleen in patients with lymphoma or certain immune diseases, such as immune thrombocytopenic purpura, etc. (page 9, lines 2-10)

- "Specific examples include antibodies and fragments against bone marrow cells, particularly hematopoietic progenitor cells, pancreatic islet cells, spleen cells, parathyroid cells, uterine endometrium, ovary cells, testicular cells, thymus cells, B-cells, T-cells, Null cells, vascular endothelial cells, bile duct cells, gall bladder cells, prostate cells, hormone receptors such as of FSH, LH, TSH, growth factor receptors, such as of epidermal growth factor, urinary bladder cells, and vas deferens cells" (page 12, lines 12-20), and

- "Antibodies that target the spleen well include the LL2 (also known as EPB-2) monoclonal antibody, disclosed in Pawlak-Byczkowska, cancer Research, 49:4568-4577 (1989), which is directed against normal and

malignant B cells, and which can be used for treating normal spleen cells in patients with immune diseases, lymphoma, and other diseases" (page 12, lines 30-35).

3. I understand the examiner to say that the term "immune disease" would be unclear and ambiguous to a knowledgeable reader of the disclosure. In particular, the examiner states that this term might encompass disease in which the immune system is "positively (*e.g.*, autoimmune) or negatively (*e.g.*, HIV) regulated)." The terms "positively" versus "negatively" regulated have never been widely accepted by the scientific community, and current terminology, introduced in the early 2000s, refers to "disturbances in homeostasis of the immune system." There are a lot of otherwise defined diseases such as infections (HIV, other viruses, bacteria), malignant diseases (lymphoma), etc., which impair or affect the immune system. However, immune activation by all of these has a defined cause. By contrast, the classical term "immune disease," circa 1992, relates to **idiopathic** disorders of the immune system. These are the so-called classical autoimmune diseases for which the cause of immune activation was unknown.

4. As an immunologist and rheumatologist, and in the context of the entire disclosure of the above-identified application to include in particular those portions which I have identified above, I do not find this term to be unclear or ambiguous. I certainly would not understand the term to include disease in which the immune system is negatively regulated, such as HIV. The term is used in conjunction with a discussion of the use of a B-cell antibody and also in conjunction with a disclosure of the ablation of normal spleen cells and a disclosure of "certain immune diseases, such as immune thrombocytopenic purpura." In this regard, I immediately recognized that the reference in the disclosure of "antibodies that target the spleen," is a reference to a targeting of immune cells that reside in the spleen. B-cell hematologic abnormalities are a consequence of immune diseases in which the immune system is positively regulated, and immune thrombocytopenic purpura (ITP) is an example of such an immune disease. In particular, B cells differentiating into plasma cells are known to make antibodies, including the autoantibodies considered to be responsible for destroying platelets in ITP. Accordingly, I have no difficulty in ascertaining the scope of the term "immune disease" in the context of the present disclosure as referring to classical autoimmune diseases, and would not understand the term to include diseases such as HIV in which the immune system is "negatively regulated" (adopting, for the moment, the little-used and imprecise terminology employed in the Office Action). I therefore have no difficulty in determining the scope of the present claims.

5. Furthermore, after reading the specification of the above-identified application, I would understand that the applicant was in possession of a method of using B-cell antibodies generally to treat immune diseases, and not just the LL2 B-cell antibody specifically. The skilled artisan would understand that applicant's contribution to the art was the teaching that B cells generally could be used to treat immune diseases. The skilled artisan would not need to know the structure of particular B-cell antibodies in order to be apprised of the full scope of applicant's invention.

I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

11/28/2007
Date

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Curriculum vitae

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Postdoctoral clinical training

1990-95	Internship and Residency of Internal Medicine, Charite Berlin, Board Certification as Doctor of Internal Medicine 1995 (Head: Prof. Dr. Gerd R. Burmester)
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1998-2000	Fellowship Rheumatology, Charite Berlin, Board Certification as rheumatologist 2000 (Head: Prof. Dr. Gerd R. Burmester)
1998-	Group Leader of the molecular immunology group at the Dept. of Medicine Charite/Rheumatology and Clinical Immunology
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1999-2003	Head of the Day care clinic/Dept. Rheumatology Charite Berlin
2001	Board certification for Transfusion Medicine, Immunology
2003	Head of the Division of Rheumatology/Dept. Medicine, Ludwigs Maximilian University Munich
Since 06/2004	Head of the interdisciplinary group Clinical Hemostaseology/Rheumatology & Head of the coagulation unit

Group leader "B cell memory" at the German Research Center of Rheumatology (DRFZ Berlin)

Research fellowships

1996-1998	Postdoctoral fellowship in clinical research in rheumatology and immunology, Department of Internal Medicine/Rheumatic Disease Division at the University of Texas, Southwestern Medical Center at Dallas, USA (Fellow of Peter E. Lipsky, MD)

Awards and honors

1990	Educational grant of the German Society of Rheumatology, Hannover
1993	Grant of the Deutsche Forschungsgemeinschaft to the 57th Meeting of the American College of Rheumatology, San Antonio, TX.
1998	"Senior Scholar Award" of the American College of Rheumatology, San Diego, CA.
2000	Rudolf-Schoen Award of the "Deutsche Gesellschaft für Rheumatologie"
2003	H-Schulze Award of the German League against Rheumatism

Journals

Member of the Editorial Board "Arthritis & Rheumatism"
Member of the Editorial Board of "Arthritis Research & Therapy"
Member of the Editorial Board "Rheumatology Reviews"
Member of the Editorial Board of Global Arthritis Research Network (GARN)

Congress Organization

International Symposiums "Rheumatology and Clinical Immunology" March 23rd –25th, 2000.
Annual Meeting of the German Association of Internists, rheumatological contributions since 2000, on behalf of the German Society of Rheumatology
Annual Meeting of the German Society of Rheumatology, 2002 in Berlin, Congress secretary
EULAR Meeting 2004, member of the international congress organization, Berlin, June 2004

Patents

Detection of anti-proteasome antibodies in body fluids (Patent Nr. 197 07 343.39).

Participation in clinical trials

- Ciprofloxacin long-term efficacy in reactive arthritis

- Randomised, placebo-controled study of the use of collagen II in early arthritis
- Randomised, placebo-controled study of the use of anakinra (IL1-Ra) in RA Arthritis
- Immune globuline therapy in systemic lupus erythematosus
- Oral pilocarpin (Salagen) in Sjögren's syndrome
- Cyclosporine A- tear drops in Sjögren's syndrome
- Immune adsorption (Prosorba) in SLE
- Immune adsorption in Sjögren's syndrome
- Epratuzumab (anti-CD22 antibody therapy) in SLE (PI)
- Epratuzumab in Sjögren's syndrome (PI)
- ADORE-study (etanercept monotherapy vs. MTX + Etanercept (Wyeth-Pharma) (PI)
- ASSERT-Study infliximab in ankylosing spondylitis (Centocor) (PI)
- COXA-study celecoxib vs. diclofenac (2 x 200 mg celecoxib vs. 2 x 75 diclofenac) Pharmacia/Pfizer (PI)
- DE 018 Open-label-Study D2E7 (Humira) in AS (Abbott-Pharmaceuticals) (PI)
- DE 013/D2E7 vs MTX vs. combination therapy (early RA) (Abbott) (PI)
- EDGE II-Studie etoricoxib vs diclofenac (gastrointestinal safety of etoricoxib) MSD (PI)
- Infliximab in ankylosing spondylitis (EU study for approval of IFX) Essex Pharma (PI)
- M02-497 D2E7-Study (Adalimumab) Abbott Wiesbaden (PI)
- RABBIT-study (long term comparison DMARDs vs. biologicals (PI)

Main research interests: Targeted therapy in rheumatic and inflammatory diseases and their impact on patient outcome and mechanism of action, B cell immunology, role of B cells and plasma cell subtypes in inflammatory rheumatic diseases, principles of the break of immune tolerance in autoimmunity, coagulation abnormalities in rheumatic diseases associated with enhanced cardiovascular risks.

Berlin, November 2007
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These documents were entered into the record on December 26, 2007.

Trubion Pharmaceuticals Announces Presentation of Positive Data From Phase IIb and Re-treatment Studies With TRU-015 in Patients With Rheumatoid Arthritis

SEATTLE, Nov 08, 2007 /PRNewswire-FirstCall via COMTEX News Network/ -- Trubion Pharmaceuticals Inc. (Nasdaq: TRBN) today announced presentation of positive data from a Phase IIb study that showed that Trubion's TRU-015 for rheumatoid arthritis (RA) provided statistically significant efficacy after a single infusion of 800 mg or 1,600 mg. In addition, Trubion also announced presentation of data showing that repeat administration with TRU-015 was well tolerated and resulted in a consistent pharmacokinetic (PK) and pharmacodynamic (PD) profile. Trubion is co-developing TRU-015 with Wyeth Pharmaceuticals, a division of Wyeth (NYSE: WYE), for the treatment of rheumatoid arthritis.

These data will be presented during two poster sessions at the annual meeting of the American College of Rheumatology (ACR) on Nov. 8 and 9, 2007. The posters are available in the Events section of Trubion's Web site at http://investors.trubion.com/events.cfm.

"TRU-015's ability to significantly improve RA signs and symptoms following a single infusion could represent a new level of convenience for patients and physicians. These results also suggest that clinical responses may be maintained during B-cell recovery," said Peter Thompson, M.D., FACP, president, chief executive officer and chairman of Trubion. "We and our partner have agreed on a design for our next study that we believe could be supportive of a registration package, and we look forward to TRU-015's continued evaluation."

TRU-015 Improves RA Disease Activity in Phase II Trial (ACR Presentation L7)

On Sept. 10, 2007, Trubion announced preliminary analysis of results for its TRU-015 Phase IIb randomized, double-blind, placebo-controlled, multicenter clinical trial that included 276 patients with rheumatoid arthritis. Patients were randomized equally into five groups that received either placebo, 200 mg, 400 mg, 800 mg or 1,600 mg of TRU-015. The study was designed to evaluate the safety and efficacy of a single intravenous infusion of TRU-015 compared to placebo for a 24-week period.

Data announced previously showed the improvement in DAS-28 compared to placebo was statistically significant in the 800 mg dose group at 12 weeks and at all subsequent assessments, and in the 1,600 mg dose group at 16 weeks and at all subsequent assessments. At 24 weeks, ACR 20, 50 and 70 response rates in the 800 mg dose group were 65 percent, 26 percent and 0 percent, respectively. ACR 20, 50 and 70 response rates in the 1,600 mg dose

group were 61 percent, 13 percent and 4 percent, respectively. ACR 20, 50 and 70 response rates at 24 weeks in the placebo group were 33 percent, 9 percent and 2 percent, respectively.

At 24 weeks, significant improvement in the Health Assessment Questionnaire Disability Index (HAQ DI) was observed in the TRU-015 1,600 mg group (-0.70 v -0.37 [p=0.008]) and the 800 mg group (-0.64 v-0.37 [p=0.035]).

The HAQ DI measures patients' physical function in defined activities. Median C-Reactive Protein (CRP) improvement was 57 percent in the 1,600 mg group, 48 percent in the 800 mg group and 28 percent in the placebo group. The CRP test measures the concentration of a protein that is present during inflammatory episodes.

TRU-015 administered as a single dose was generally well tolerated, and only one subject in the 400 mg group experienced a grade 3 adverse event on the day of infusion.

Comparable Data Following Repeat Administration (ACR Presentation 309)

The objective of the re-treatment study was to evaluate the safety, PD, PK and immunogenicity of TRU-015 for RA with repeated doses after receiving initial administration in a Phase I/IIa study. Patients treated with a single course of 5 mg/kg or higher in a previously conducted TRU-015 Phase I/IIa study were eligible for re-treatment. Patients who received a single infusion of 5 mg/kg received a single infusion of 5 mg/kg upon re-treatment, and those who received higher doses of TRU-015 received a single infusion of 15 mg/kg upon re-treatment. PD response of B-cells was also evaluated after initial treatment and after re-treatment.

Fifty-four patients were eligible for re-treatment, and at the time of this analysis, re-treatment data were available for 36 patients. B-cell depletion and recovery following re-treatment with TRU-015 was comparable to that seen after initial treatment. Ongoing patient evaluations showed maintenance of ACR responses with repeated single doses of TRU-015 at six-month intervals through at least two retreatment courses. Total serum IgG levels remained within normal limits. In addition, subjects treated with three or more courses of therapy experienced persistent decreases in rheumatoid factor and IgM levels without experiencing decreases in IgG or IgA levels. No neutralizing antibodies to TRU-015 had been detected at the time of this assessment.

Re-treatment with TRU-015 was generally well tolerated, and no grade 3 or 4 adverse events occurred on the day of infusion.

Research article

Open Access

Initial clinical trial of epratuzumab (humanized anti-CD22 antibody) for immunotherapy of systemic lupus erythematosus

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Abstract

B cells play an important role in the pathogenesis of systemic lupus erythematosus (SLE), so the safety and activity of anti-B cell immunotherapy with the humanized anti-CD22 antibody epratuzumab was evaluated in SLE patients. An open-label, single-center study of 14 patients with moderately active SLE (total British Isles Lupus Assessment Group (BILAG) score 6 to 12) was conducted. Patients received 360 mg/m^2 epratuzumab intravenously every 2 weeks for 4 doses with analgesic/antihistamine premedication (but no steroids) prior to each dose. Evaluations at 6, 10, 18 and 32 weeks (6 months post-treatment) follow-up included safety, SLE activity (BILAG score), blood levels of epratuzumab, B and T cells, immunoglobulins, and human anti-epratuzumab antibody (HAHA) titers. Total BILAG scores decreased by ≥ 50% in all 14 patients at some point during the study (including 77% with a ≥ 50% decrease at 6 weeks), with 92% having decreases of various amounts continuing to at least 18 weeks (where 38% showed a ≥ 50% decrease). Almost all patients (93%) experienced improvements in at least one BILAG B- or C-level disease activity at 6, 10 and 18 weeks. Additionally, 3 patients with multiple BILAG B involvement at baseline had completely resolved all B-level disease activities by 18 weeks. Epratuzumab was well tolerated, with a median infusion time of 32 minutes. Drug serum levels were measurable for at least 4 weeks post-treatment and detectable in most samples at 18 weeks. B cell levels decreased by an average of 35% at 18 weeks and remained depressed at 6 months post-treatment. Changes in routine safety laboratory tests were infrequent and without any consistent pattern, and there was no evidence of immunogenicity or significant changes in T cells, immunoglobulins, or autoantibody levels. In patients with mild to moderate active lupus, 360 mg/m^2 epratuzumab was well tolerated, with evidence of clinical improvement after the first infusion and durable clinical benefit across most body systems. As such, multicenter controlled studies are being conducted in broader patient populations.

Introduction

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that can involve many organ systems [1]. In Europe and the United States, estimates of the number of affected individuals range from 24 to 65 cases per 100,000 people [1,2]. The clinical course of SLE is episodic, with recurring activity flares causing increasing disability and organ damage. Cyclophosphamide, azathoprine, and corticosteroids remain important for long-term management of most patients having active disease, and even those in clinical remission [1]. Despite the important advances made with these drugs, especially cyclophosphamide, in controlling lupus disease activity, they have considerable cytotoxicity and cause, for example, bone marrow depression, ovarian failure, enhanced risk of bladder cancer, as well as the known side effects of long-term systemic corticosteroid therapy. As such, there continues to be a need for the development of targeted and less toxic therapies.

BCR = B cell antigen receptor; BILAG = British Isles Lupus Assessment Group; HACA = human anti-chimeric antibody; HAHA = human anti-human (epratuzumab) antibody; NCI CTC = National Cancer Institute Common Toxicity Criteria; NHL = non-Hodgkin lymphoma; SLE = systemic lupus erythematosus.

Specific autoantibodies against nuclear, cytoplasmic, and membrane antigens remain the serological hallmark of SLE. While lymphopenia is common, there is an increase in the level of activated B cells [3,4] and characteristic alterations of B cell subpopulations [5,6] that may be driven by extrinsic or intrinsic factors. B cells appear to have a key role in the activation of the immune system, in particular through the production of cytokines and by serving as antigen-presenting cells (reviewed recently in [7]). Although B cell activation can occur independently of T cell help in lupus, a substantial fraction of B cells is activated in a T cell dependent manner [8-10], as demonstrated by isotype switching and affinity maturation of B cells [11,12] and enhanced CD154-CD40 interactions [13]. Useful insight into the pathogenesis of lupus has been obtained with animal models. MRL/lpr mice spontaneously develop a lupus-like autoimmune disease in an age-dependent manner, including autoantibody production, arthritis, skin lesions, and severe nephritis, which usually leads to early demise from renal failure [14]. When rendered B cell deficient, they no longer develop nephritis, mononuclear infiltrates are no longer detectable in the kidneys or skin, the number of activated memory T cells are markedly reduced, and infusions of pooled serum from diseased MRL/lpr mice lead to glomerular antibody deposition, but not the development of renal disease [15,16]. However, when reconstituted with B cells not able to secrete circulating antibodies, they develop nephritis and vasculitis [17]. As such, it appears that B cells play a direct role in promoting disease beyond the production of autoantibodies [18].

Depleting B cells with anti-CD20 monoclonal antibodies has emerged as a potentially new therapeutic strategy for certain autoimmune diseases. The chimeric monoclonal antibody rituximab depletes B cells by targeting the pan-B cell surface antigen CD20. Preliminary experience with rituximab in about 100 patients with SLE (recently reviewed in [7]) and other autoimmune diseases has been encouraging [6,19-22].

Due to the central role of B cells in the pathogenesis of certain autoimmune diseases, targeted anti-B cell immunotherapies would be expected to offer therapeutic value in the setting of SLE. In addition to CD20, another unique target is CD22, a 135 kDa glycoprotein that is a B-lymphocyte-restricted member of the immunoglobulin superfamily, and a member of the sialoadhesin family of adhesion molecules that regulate B cell activation and interaction with T cells [23-27]. CD22 has seven extracellular domains and is rapidly internalized when cross-linked with its natural ligand, producing a potent costimulatory signal in primary B cells [25,28-30]. The function of CD22 in cell signaling is suggested by six tyrosine and three inhibitory domain sequences in the intra-cellular cytoplasmic tail. These inhibitory domains are phosphorylated by the nonreceptor kinase Lyn upon B cell antigen receptor (BCR) activation by IgM ligation, leading to the activation and recruitment of SHP-1 phosphatase [31,32]. SHP-1 is a tyrosine phosphatase that negatively regulates several intracellular signaling pathways, including the calcium pathway, through dephosphorylation of signaling intermediates, such as Lyn and Syk. CD22 is first expressed in the cytoplasm of pro-B and pre-B cells, and then on the surface of B cells as they mature, with expression ceasing with B cell differentiation into plasma cells [23]. Studies in CD22-deficient mice and in CD22-negative cell lines have shown an increase in calcium response to BCR ligation [33-36], indicating that CD22 inhibition of BCR signaling is achieved through the mechanism of controlling calcium efflux in B cells. It has been reported that this effect of CD22 is mediated by potentiation of plasma membrane calcium-ATPase and requires SHP-1 [37]. Animal experiments indicate that CD22 plays a key role in B cell development and survival, with CD22-deficient mice having reduced numbers of mature B cells in the bone marrow and circulation, and with the B cells also having a shorter life span and enhanced apoptosis [31].

Therefore, CD22 is an attractive molecular target for therapy because of its restricted expression; it is not exposed on embryonic stem or pre-B cells, nor is it normally shed from the surface of antigen-bearing cells. Initially, a mouse monoclonal antibody (mLL2, formerly EPB-2) was developed and characterized that specifically binds to the third domain of CD22 [38,39]. Immunohistological evaluation revealed that it recognized B cells within the spleen and lymph nodes, but did not react with antigen unrelated to B cells in normal and solid tumor tissue specimens, and flow cytometry showed no reactivity with platelets, red blood cells, monocytes, and granulocytes in normal peripheral blood [38,39]. The complementarity-determining regions of mLL2 were subsequently grafted onto a human IgG_1 genetic backbone [40]. Epratuzumab, the resulting complementarity-determining region-grafted (recombinant) 'humanized' monoclonal antibody (hLL2), is 90% to 95% of human origin, thus greatly reducing the potential for immunogenicity. Epratuzumab has been shown to mediate antibody-dependent cellular cytotoxicity *in vitro*[41] , and may also exhibit biological activity through modulating BCR function (J Carnahan, R Stein, Z Qu, K Hess, A Cesano, HJ Hansen, DM Goldenberg, manuscript submitted).

In clinical trials, over 400 patients with non-Hodgkin lymphoma (NHL) or other B cell malignancies have received epratuzumab administered as 4 consecutive weekly infusions over about 60 minutes. An initial phase I/II study administered doses of up to 1,000 mg/m^2, with patients premedicated each week with oral acetaminophen and diphenhydramine to minimize potential infusion reactions. Epratuzumab toxicity consisted primarily of mild to moderate transient infusion-related events during the first infusion, and only one patient with a prior right lung resection for a fungal abscess had a serious event (bronchospasm during infusion), which was treated with parenteral medications. Based on this safety record, objective evidence of tumor

response, and less severe depression of circulating B cells [42,43], 4 consecutive weekly doses of 360 mg/m^2 epratuzumab was selected as a sufficiently safe and efficacious treatment regimen to warrant further clinical development. A pharmacokinetic analysis of weekly dosing subsequently demonstrated that the post-treatment serum half-life of epratuzumab in NHL patients was 19 to 25 days, consistent with the half-life of a human IgG_1 [44]. As such, a longer interval between doses was indicated, and a biweekly dosing schedule was selected for this initial study in SLE. We report here the first experience of treating an autoimmune disease with a CD22 antibody, epratuzumab.

Materials and methods

This initial, phase II, open-label, non-randomized, single-center study was undertaken to obtain preliminary evidence of therapeutic activity in SLE, to confirm the safety, tolerance and lack of immunogenicity of epratuzumab in this population, and to evaluate pharmacokinetic and pharmacodynamic parameters. The study was approved by the Ethics Committee of Charité University Hospital.

Patient population

Males or non-pregnant, non-lactating females, ≥ 18 years of age, were eligible to participate provided they had a diagnosis of SLE according to the American College of Rheumatology revised criteria (fulfilled ≥ 4 criteria), with SLE for at least 6 months, and at least one elevated autoantibody level (antinuclear antibodies/ANA and/or anti-dsDNA) and moderately active disease (a score of 6 to 12 for total British Isles Lupus Assessment Group (BILAG) disease activity) at study entry. Patients were excluded if they had prior rituximab or other antibody therapy, allergies to murine or human antibodies, experimental therapy within 3 months, active severe CNS (central nervous system) lupus, laboratory abnormalities (hemoglobin < 8.0 g/dl, WBC (white blood cells) < 2,000/mm^3, ANC (absolute neutrophil cells) < 1,500/mm^3, platelets < 50,000/μl, liver transaminases or alkaline phosphatase more than twice upper limit of normal, serum creatinine > 2.5 mg/dl, or proteinuria > 3.5 gm/day), thrombosis, drug or alcohol abuse, infection requiring hospitalization within 3 months, long-term active infectious diseases (tuberculosis, fungal infections) within 2 years, malignancy (except basal cell carcinoma, cervical carcinoma in situ (CIS), history of recurrent abortions (2 or more), or known HIV, hepatitis B or C, or other immunosuppressive states.

Concomitant medications

Pulsed methylprednisolone, other high-dose corticosteroids, cyclophosphamide, and intravenous, joint, or intramuscular corticosteroid injections were not allowed during the study or within four weeks of study entry. Low-dose corticosteroids (prednisone, = 20 mg/day or equivalent) or background therapy with standard antirheumatic immunosuppressives (for example, azathioprine, methotrexate) was permitted provided there were no dosing changes during the study or within four weeks prior to study entry. Antimalarials, non-steroidal anti-inflammatory drugs (NSAIDs), ACE-inhibitors or angiotensin receptor antagonists were also allowed, provided there were no dosing changes during the study or within two weeks of study entry.

Treatment schedule

After satisfying eligibility, signing informed consent, and undergoing baseline evaluations, all patients received 4 doses of 360 mg/m^2 epratuzumab administered every other week with paracetamol (acetaminophen) and an antihistamine (but no steroids) given as premedication prior to each dose.

Study evaluations

The BILAG system was used to categorize the severity level of lupus disease activity in each patient at study entry and at post-treatment evaluations obtained at 6 (24 hours after the last infusion), 10 and 18 weeks and at an additional 32 weeks (6 month post-treatment) follow-up visit. The BILAG system organizes lupus-associated signs and symptoms according to eight body systems: general/constitutional, mucocutaneous, neurological, musculoskeletal, cardiovascular/respiratory, vasculitic, renal, hematological domains [45,46]. At each evaluation, the presence and change of any signs and symptoms were recorded and the level of any disease activity within each body system determined on a treatment-intent basis, according to BILAG rules as: A (severely active disease sufficient to require disease-modifying treatment, for example, > 20 mg/d prednisolone, immunosuppressants/cytoxics); B (moderately active disease requiring only symptomatic therapy, for example < 20 mg/d prednisolone, antimalarials, NSAIDs alone or in combination); or C (stable mild disease with no indication for changes in treatment). To assign an overall disease activity level for each patient, a total BILAG score was determined by adding a numerical severity score (A = 9, B = 3, C = 1, no activity = 0) across the eight body systems. Other evaluations at these times included an SLE panel (autoantibodies, C3, C-reactive protein/CRP, erythrocyte sedimentation rate/ESR, other laboratory tests), vital signs, physical examination, adverse events, routine safety laboratory tests (hematology, serum chemistry), urinalysis, serum immunoglobulins, peripheral blood B and T cells, epratuzumab serum levels (analyzed by sponsor), and human anti-human (epratuzumab) antibody titers (HAHA; analyzed by sponsor).

Human anti-human (epratuzumab) antibody assay

The sponsor's HAHA test is a competitive ELISA assay, where the capture reagent is epratuzumab and the probe is an anti-epratuzumab-idiotype antibody. The anti-idiotype antibody is an acceptable surrogate for what is reacted against in an immunogenic response by humans against the binding portion of epratuzumab that distinguishes the molecule from other human antibodies (for instance, the framework region that has human amino acid sequences). Test results are derived from

Table 1

Number of patients with B-level disease activity at study entry in each BILAG body system

Body system	Number of patients	Contributing signs/symptoms* (number of patients)
I. General/ constitutional	3	Fatigue/malaise/lethargy (3)
		Anorexia/nausea/vomiting (2)
		Unintentional weight loss > 5% (1)
II. Mucocutaneous	13	Malar erythema (11)
		Active localized discoid lesions (2)
		Mild maculopapular eruption (1)
III. Neurological	0	
IV. Musculoskeletal	2	Arthritis (2)
V. CV/Respiratory	2	Dyspnea (2)
		Pleuropericardial pain (2)
VI. Vasculitis	5	Minor cutaneous vasculitis (nailfold/digital vasculitis, purpura, urticaria) (5)
VII. Renal	0	
VIII. Hematology	1	Anemia (hemoglobin < 11 g/dL) (1)

*Signs and symptoms that contributed to the B-level disease activity according to BILAG rules.

an eight-point standard curve with varying dilutions of anti-idiotype antibody in bovine serum albumin. Patient serum samples are diluted 1:2 with bovine serum albumin and assayed in triplicate. The anti-idiotype standard curve is used to determine the presence of HAHA in unknown samples. An acceptable assay is based on linear regression parameters that must be met to define a valid assay.

Statistical analyses

The primary assessment of disease activity compared post-treatment BILAG results with those at study entry, using total BILAG scores for overall assessment and letter grade categories to assess the level of disease activity within each body system. Adverse events and safety laboratory tests were graded according to NCI CTC version 3.0 criteria on a 1 to 4 scale for toxicity (1, mild; 2, moderate; 3, severe; 4, life threatening). All analyses of efficacy, safety, tolerance, immunogenicity, pharmacokinetics, and pharmacodynamics used descriptive statistics. Wilcoxon signed rank test was used to assess the statistical significance of changes in total BILAG scores compared to their baseline values. All statistical tests used a significance level of 0.05.

Table 2

Number of patients with C-level disease activity at study entry in each BILAG body system

Body system	Number of patients	Contributing signs/symptoms* (number of patients)
I. General/ Constitutional	11	Fatigue/malaise/lethargy (10)
		Anorexia/nausea/vomiting (1)
		Lymphadenopathy/splenomegaly (1)
		Pyrexia (documented) (1)
II. Mucocutaneous	1	Mild alopecia (1)
III. Neurological	10	Episodic migrainous headaches (8)
		Severe, unremitting headache (2)
IV Musculoskeletal	11	Arthralgia (10)
		Myalgia (9)
		Improving arthritis (1)
V. CV/ Respiratory	2	Dyspnea (1)
		Pleuropericardial pain (1)
VI. Vasculitis	4	Raynaud's (3)
		Livido reticularis (1)
VII. Renal	4	Mild/stable proteinuria (4)
VIII. Hematology	11	Lymphocytopenia (< 1500 cells/μl) (10)
		Evidence of circulating anticoagulant (1)
		Decreased platelets (< 150,000/μl) (1)

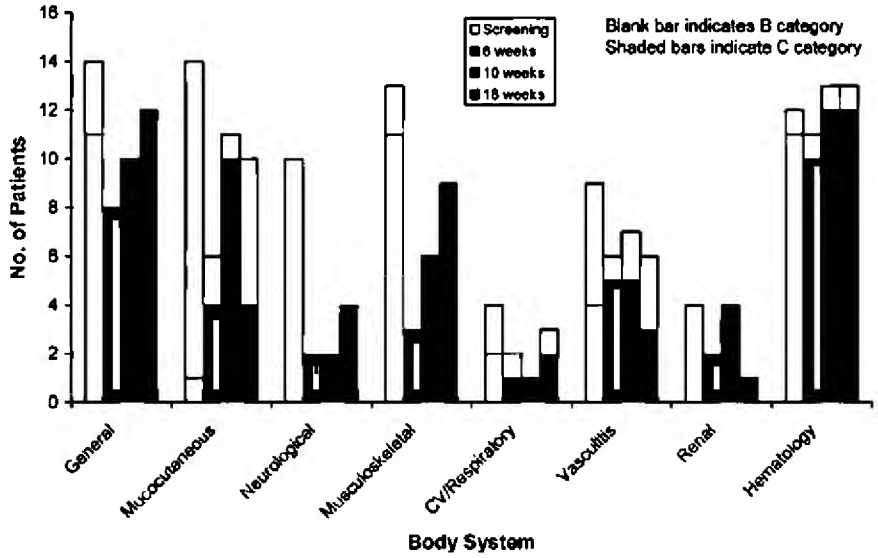
*Signs and symptoms that contributed to the C-level disease activity according to BILAG rules.

Results

Demographics and patient characteristics at study entry

A total of 14 Caucasian patients (13 females and 1 male; 23 to 53 years old, median age 40 years) were enrolled. At study entry, the patients had been initially diagnosed with SLE 1 to 19 years (median 10 years) earlier and were receiving corticosteroids ($n = 13$, 1 to 12 mg/day prednisolone) plus immunosuppressives ($n = 11$, including 50 to 200 mg/day azathioprine, $n = 9$; 20 mg/day methotrexate, $n = 2$; 2 g/day mycophenalate mofetil, $n = 1$), and antimalarials ($n = 6$, 200 to 600 mg/day hydroxychloroquine). All patients had positive ANA at study entry (titers of 80:1 to 5,120:1), and 5 patients (36%) had positive anti-dsDNA antibody levels (> 10 U/ml). Ten patients (71%) had ESR values that were elevated (> 15 mm/h) and 4 patients (29%) had raised CRP levels (> 0.5 mg/dl), while only 3 patients (21%) had C3 levels that were borderline low or decreased (< 90 mg/dl), and no patient had

Figure 1



Frequency comparison of BILAG B- and C-level activities for each body system at screening, 6, 10 and 18 weeks.

positive direct Coombs' or serum haptoglobulin levels elevated above borderline.

All patients had total BILAG scores of 6 to 12 (median 10) at study entry. No patient had A-level disease activity in any body system, 13 patients had B-level disease activity in at least one body system (2 with three Bs, 9 with 2 Bs, 2 with one B) and one patient had only C-level activities. B-level disease occurred primarily in the mucocutaneous, vasculitis, and general/constitutional body systems, with no B-level disease activity in the neurological or renal systems (Table 1), while C-level disease occurred primarily in the general/constitutional, musculoskeletal, hematological and neurological body systems (Table 2). The actual signs and symptoms at study entry that contributed to the B-level disease activity according to the BILAG rules are also summarized in Table 1, while those contributing to C-level disease activity are summarized in Table 2.

Study drug administration

Twelve of the 14 patients (86%) completed all 4 infusions of 360 mg/m^2 epratuzumab as scheduled, while one patient with sleepiness attributed to premedication IV antihistamines prematurely terminated the first infusion but subsequently completed all 3 remaining infusions without further event, and one patient completed the first two infusions, but discontinued further infusions after development of herpes zoster, which responded to antivirals. The infusions were well tolerated, with a median infusion time of 32 minutes (23 to 86 minutes), and with infusion reactions in 6 patients all limited to occurrences of transient, mild (grade 1 NCI toxicity) adverse events (flu-like symptoms, tracheitis/throat ache, $n = 2$; arthralgia/myalgia, fever, fatigue, nausea, headache, chills, or rash, $n = 1$).

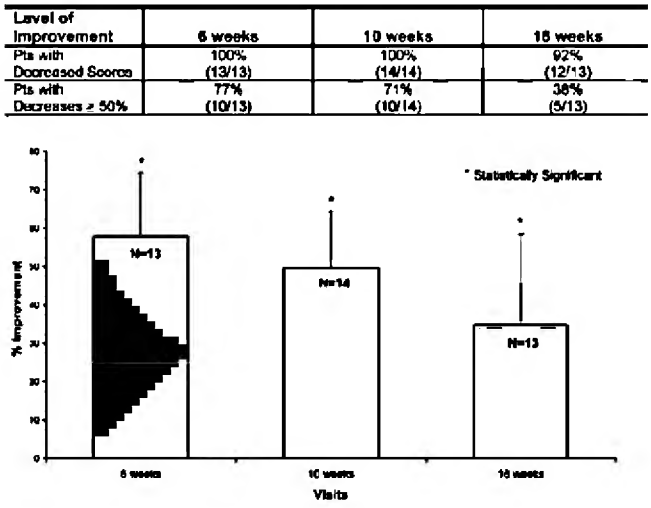
Post-treatment evaluations and follow-up

All patients remained in the study through the 18-week post-treatment evaluation period. One patient had a late 18-week visit that fell within the 32-week time frame and the corresponding data were hence re-assigned to the 32-week visit. The single patient who did not complete all 4 infusions continued to receive post-treatment evaluations beginning at the 10-weeks follow-up visit. Except for the aforementioned deviations, all patients received post-treatment evaluations at 6, 10, and 18 weeks. One patient was lost to follow-up after 18 weeks, while 13 patients returned for the final 32-week evaluations (8 patients as scheduled, 5 with a delayed visit between 42 to 82 weeks).

BILAG treatment response

The effect of epratuzumab on clinical manifestations was evaluated at 6, 10, and 18 weeks using numerical total BILAG scores as well as categorical scores. The compositions of B- and C-level activities improved after treatment, primarily in the general, mucocutaneous and musculoskeletal systems (Figure 1). Improvement in C-level activity was also observed in the neurological and renal domains. Improvements in the general, mucocutaneous, neurological and musculoskeletal systems occurred earlier compared to the cardiovascular/respiratory, vasculitic and renal systems (Figure 2). However, the limited number of patients with manifestations in each of these systems precludes a definitive determination of preferential effects. In terms of changes in the total BILAG score, statistically significant improvement was observed at 6, 10, and 18 weeks (Figure 3). Additionally, a substantial proportion of patients showed 50% or more improvement in total BILAG score at weeks 6, 10, and 18 (77%, 71% and 38%, respectively). At the final 32-week evaluation, statistically significant

Figure 2



Overall frequency and mean improvement of total disease activity as measured by the total BILAG score at 6, 10 and 18 weeks.

improvement in total BILAG score continued to be observed, with 15% of the patients achieving 50% or more improvement.

In a separate analysis, the total number of patients who achieved BILAG improvements in the particular domains at 6, 10 and 18 weeks of follow-up are summarized in Table 3. This indicates that the most characteristic BILAG domains, as also seen in Figure 2, were more likely to respond, although the duration of response was very similar throughout the domains. In fact, deterioration in BILAG categorical scores compared to baseline was infrequently seen during the study (Table 4). Only two patients (14%) showed worsening of hematological

Table 3

Number of patients with improvement from baseline BILAG B- and C-level activities

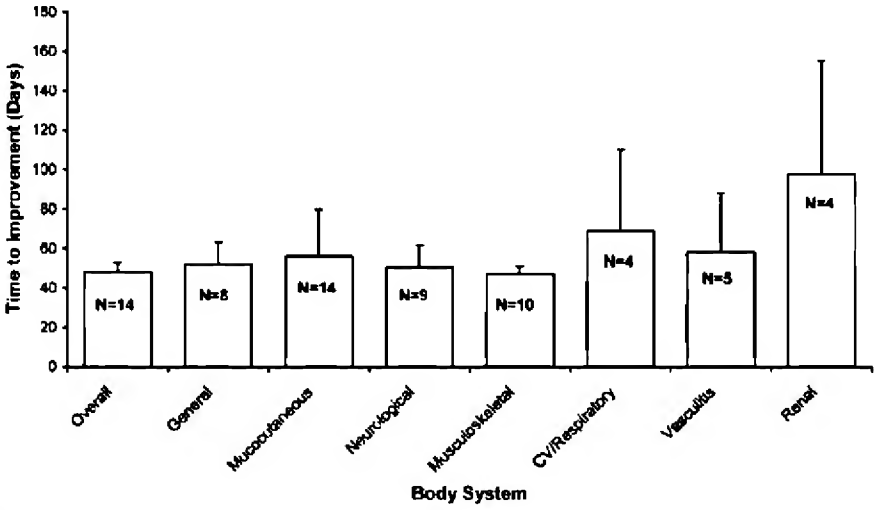
BILAG body system	6 weeks[a]	10 weeks	18 weeks
General ($N = 14$)[b]	6 (43%)	5 (36%)	2 (14%)
Mucocutaneous ($N = 14$)	11 (79%)	8 (57%)	6 (43%)
Neurological ($N = 10$)	7 (70%)	8 (80%)	6 (60%)
Musculoskeletal ($N = 13$)	9 (69%)	7 (54%)	4 (31%)
CV/Respiratory ($N = 4$)	3 (75%)	3 (75%)	3 (75%)
Vasculitis ($N = 9$)	4 (44%)	3 (33%)	3 (33%)
Renal ($N = 4$)	2 (50%)	1 (25%)	3 (75%)
Hematology ($N = 12$)	0 (0 %)	0 (0 %)	0 (0 %)
Overall[c] ($N = 14$)	13 (93%)	14 (100%)	13 (93%)

[a]Twenty-four hours after fourth infusion. [b]N = number of patients with involvement in a particular body system at entry. [c]As applied to any BILAG body system.

parameters (lymphocytopenia), one starting at 6 weeks and the other at 18 weeks. Another patient manifested renal (mild proteinuria) deterioration at 10 weeks. Overall, at week 18, 3 patients (21%) had a deteriorated BILAG assessment in at least one body system compared to baseline.

An additional analysis was performed to determine the durability of resolution of certain B- and C-level activities (Table 5). Although in a number of patients, B- and C-level activities resolved persistently, the heterogeneity of patients' manifesta-

Figure 3



Mean time to improvement of each BILAG body stystem. Mean time to improvement (in days) of each BILAG body system during the follow-up of the study (N denotes the number of patients available for analysis for each body system). Since the first evaluation was scheduled for 6 weeks, the earliest time to improvement is at least 42 days.

tions again precluded the identification of a preferential response profile to the drug.

Safety

During or following treatment, a total of ten patients reported adverse events. As reported above, six had mild, transient, infusional reactions and one patient experienced somnolence following antihistamine medication. Subsequently, five patients had infections (including herpes zoster, otitis media, *Helicobacter pylori*-associated gastritis, vaginitis/vaginal candidiasis, cystitis, and tonsillitis) that resolved with appropriate treatment, and one patient had spinal contusion from a traffic accident. Standard safety laboratory tests showed no consistent pattern of change from baseline, and infrequent post-treatment increases in NCI CTC v3.0 toxicity grades for these laboratory tests were all limited to changes of one grade level except for one patient with an increase in lymphocytes from grade 1 to grade 3, and another from grade 0 to grade 3 (Table 6).

Pharmacokinetics and immunogenicity

Of the 14 patients, serum samples for analysis of pharmacokinetics and immunogenicity (HAHA) by ELISA assay were collected in a limited number of patients post-treatment at 6 weeks (n = 12), 10 weeks (n = 7) and 18 weeks (n = 7). Epratuzumab serum levels were measurable in all available samples through at least 10 weeks post-treatment and were still detectable above the 0.5 μg/ml assay limit in 5/7 samples evaluated at 18 weeks, with median values of 120 μg/ml (range 49 to 350) at 6 weeks, 48 μg/ml (range 31 to 138) at 10 weeks, and 8.3 μg/ml (range 1.82 to 25) at 18 weeks. Figure 4 shows the individual measurements over time. There was a single sample showing 1.42 μg/ml at 32 weeks. HAHA analysis gave no evidence of immunogenicity, with all post-treatment values either remaining below the 50 ng/ml sensitivity of the assay or not increased from baseline values prior to treatment.

Table 4

Number of patients with deteriorating BILAG activities from baseline

BILAG body system (N = 14)[a]	6 weeks[b]	10 weeks	18 weeks
General	0 (0 %)	0 (0 %)	0 (0 %)
Mucocutaneous	0 (0 %)	0 (0 %)	0 (0 %)
Neurological	0 (0 %)	0 (0 %)	0 (0 %)
Musculoskeletal	0 (0 %)	0 (0 %)	0 (0 %)
CV/Respiratory	0 (0 %)	0 (0 %)	1 (7 %)
Vasculitis	0 (0 %)	0 (0 %)	0 (0 %)
Renal	0 (0 %)	1 (7 %)	0 (0 %)
Hematology	1 (7 %)	1 (7 %)	2 (14%)
Overall[c]	1 (7 %)	2 (14%)	3 (21%)

[a]N = total number of patients. [b]Twenty-four hours after fourth infusion. [c]As applied to any BILAG body system.

Immunology laboratory tests

Table 7 shows that at the first evaluation after treatment, mean B cell levels decreased by 35% and persisted at these levels on subsequent evaluations (Figure 5), with no evidence of onset of recovery by the final study evaluation at 32 weeks (6 months post-treatment). In contrast, there does not appear to be any consistent pattern of decreases/increases in T cell levels or serum levels of IgG, IgA, or IgM following treatment (Table 7).

Although all 14 patients had measurable ANA titers (1:80 to 1:5,120) at study entry, no patient had consistent post-treatment decreases, including evaluations at 32 weeks (6 months post-treatment) follow-up (8 patients had no changes at any evaluation, 5 doubled their baseline titers at one or more evaluations, and one patient had an isolated decrease at one evaluation). Five patients had elevated anti-dsDNA antibodies (10 to 123 U/ml) at study entry, but none had any decreased post-treatment values (2 patients had no significant changes, and 3 had increases at one or more evaluations). C3 levels that were decreased or borderline for 3 patients at study entry remained virtually unchanged post-treatment, as did mean C3 values for all patients.

Table 5

Number of patients in each BILAG body system with resolution of baseline B- and C-level disease activities

Body system	B level	C level
General	3/3 (100%)	0/11 (0%)
Mucocutaneous	4/13 (31%)	0/1 (0%)
Neurological	0/0	2/10 (20%)
Musculoskeletal	1/2 (50%)	1/11 (9%)
CV/Respiratory	0/2 (0%)	2/2 (100%)
Vasculitis	2/5 (40%)	0/4 (0%)
Renal	0/0	2/4 (50%)
Hematology	0/1 (0%)	0/11 (0%)

Resolution is defined as post-treatment improvement of baseline disease activity level by at least one category level (B to C, D, or E; C to D or E) at one or more evaluations up to 18 weeks, with no categorical deterioration from the baseline activity level prior to improvement, and no reversion to the baseline activity level once any improvement has occurred. Additionally note that 3 patients with multiple BILAG B involvement at baseline had completely resolved all B-level disease activities by 18 weeks.

Table 6

Post-treatment increases in NCI CTC v3.0 toxicity grades from baseline values

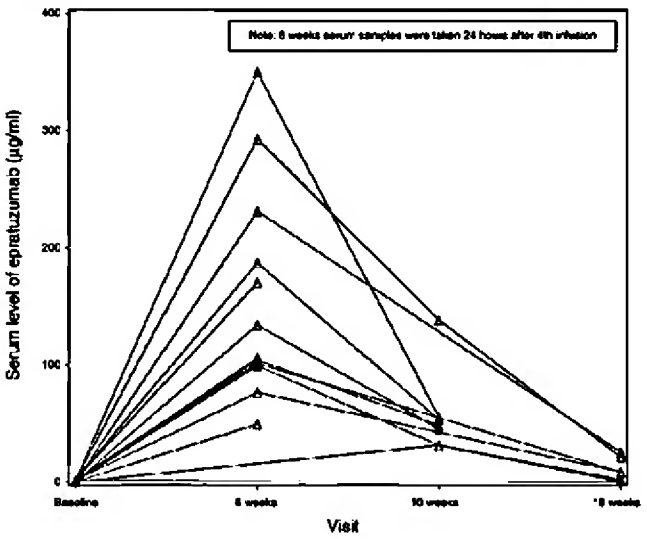
Labparameter	No increase	Toxicity increase	
		1 grade	2–3 grades
Hematology			
Hemoglobin	10	4	0
Platelets	12	2	0
WBC	11	3	0
ALC	6	6	2
ANC	13	1	0
Chemistry			
Creatinine	10	4	0
Total Bilirubin	14	0	0
Alkaline phosphatase	12	2	0
ALT (SGPT)	9	5	0
AST (SGOT)	10	4	0
GGT	12	2	0

ALC, absolute lymphocyte count, ANC, absolute neutrophil count, ALT, alanine aminotransferase, AST, aspartate aminotransferase, GGT, gamma glutamyl transferase, WBC, white blood cell

Discussion

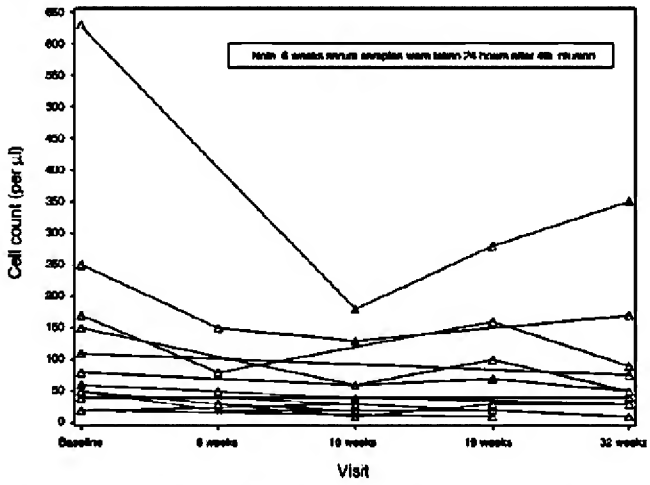
The pathogenesis of SLE remains enigmatic, but a central feature of this disease is the loss of immune tolerance and enhanced B cell activity. Although the number of B cells in the peripheral blood is often decreased, those that are present show characteristic alterations and have abnormal phenotypes indicative of activation [5,47]. Therefore, B cell depletion is an attractive therapeutic strategy for patients with SLE. The availability of the chimeric anti-CD20 antibody rituximab (Rituxan® Genentech, South San Francisco, CA, USA; Biogen Idec, Boston, MA, USA) made it possible to test this hypothesis.

Figure 4



Serum levels of epratuzumab as detected by ELISA in the patients during the study.

Figure 5



Follow-up of peripheral B cell levels during the study among individual study patients.

Initially, Isenberg and coworkers [19] treated 6 patients with active and otherwise refractory SLE (median BILAG score 14, range 9 to 27) with rituximab given in 500 mg doses 2 weeks apart with 2 doses of 750 mg iv cyclophosphamide and oral prednisolone cover (30 or 60 mg for 5 days). The treatment was safe and well tolerated, B cell depletion occurred, and BILAG total scores improved at 6 months (median 6, range 3 to 8). Looney and colleagues [6] initiated an open-label rituximab study of 17 patients with SLE (≥ 6 systemic lupus activity measurement, SLAM score) who were treated with either one 100 mg/m^2 dose, one 375 mg/m^2 dose, or four 375 mg/m^2 doses. Oral prednisone (40 mg for two doses) also was administered. B cell decreases were variable, with a 35% mean decrease persisting over the 6-month observation period, and clinical efficacy was demonstrated in patients with B cell depletion. Less than 6/17 of their patients developed human anti-chimeric antibody (HACA) at a level higher than or equal to 100 ng/ml when treated with this protocol.

All of these studies and case reports have so far been of short duration [7,48]. Usually, the B cell depletion in SLE is profound, as in patients with NHL, but shorter lasting. Therefore, it is very likely that cyclical therapy will be needed to provide long-term benefit for patients with SLE. While the immunogenicity of rituximab has not been clinically important (HACA < 1%) for the management of patients with NHL, approximately 4% of patients with rheumatoid arthritis developed HACA and 8% to 10% with SLE did so also, in spite of being

Table 7

Post-treatment changes of lymphocytes and immunoglobulins

	Baseline values and post-treatment percent change from baseline (mean ± SD)				
	Baseline	6 weeks	10 weeks	18 weeks	32 weeks
Lymphocytes	*N* = 14	*N* = 6	*N* = 8	*N* = 9	*N* = 11
B cells	123 ± 160 cells/μl	-35% ± 23%	-41% ± 41%	-34% ± 23%	-44% ± 21%
T cells	744 ± 554 cells/μl	+16% ± 80%	+28% ± 78%	+47% ± 109%	+17% ± 69%
Immunoglobulins		*N* = 12	*N* = 14	*N* = 10	*N* = 11
IgG	1,252 ± 355 mg/dl	+3% ± 8%	+5% ± 13%	+5% ± 9%	1% ± 13%
IgA	226 ± 94 mg/dl	+3% ± 11%	+8 ± 13%	+5% ± 12%	+10% ± 20%
IgM	117 ± 73 mg/dl	-12% ± 18%	-1% ± 23%	-6% ± 19%	-9% ± 9%

SD, standard deviation.

treated with various doses of steroids and/or cytotoxic agents in combination with rituximab. Thus, a less immunogenic antibody (for example, a human or humanized form) is likely needed in the management of patients with autoimmune diseases, since it is expected that repeated dosing will be required in patients with such chronic diseases.

This initial study demonstrated that 360 mg/m^2 epratuzumab, a humanized CD22-specific monoclonal antibody, administered every other week for a total of 4 doses was safe and well-tolerated in SLE patients, with few significant adverse events, alterations of standard safety laboratory tests, and no evidence of immunogenicity. In addition to the minimal infusion reactions, the ability to complete an infusion within approximately 0.5 to 1 hour and the lack of immunogenicity are also likely to be more important treatment considerations in autoimmune diseases, as mentioned previously.

With this dosing schedule, virtually every patient with moderate disease activity (total BILAG score of 6 to 12) demonstrated symptomatic improvement using BILAG total scores. The BILAG total score results indicate that 77% of the patients achieved a ≥ 50% decrease in their overall disease activity at 6 weeks follow up. Furthermore, most patients (92%) continued to show reduced disease activity for at least 18 weeks, and even 38% showed a sustained response with BILAG reductions of 50% or more compared to study entry. Since this first study considered moderately active lupus patients with BILAG total scores of 6 to 12, the resulting heterogeneity precludes the identification of any preferential effect on one or the other BILAG domains as shown from different perspectives of efficacy analysis.

In addition to treating mild BILAG C-level symptoms, epratuzumab immunotherapy reduced all BILAG B-level activity in the majority of patients presenting with more serious disease, including patients with B-level activity in several body systems. The current data limit the conclusions that can be drawn regarding therapeutic effects for some systems, such as B-level disease in the neurological and renal systems, and only one case of lymphopenia in the hematological system showed improvement. In spite of small numbers, CD22-immunotherapy with epratuzumab appeared to be effective for treating disease in many of the other body/organ systems.

Although the biweekly dosing schedule used in this study demonstrated apparent activity, the serum levels of antibody measured here appear to be less than those in studies of NHL, where a weekly schedule of dose administrations has shown antitumor activity [42-44]. Hence, other dosing schedules in future clinical trials are warranted to assess the effects of increasing the serum levels of epratuzumab.

Compared to the complete depletion of B cells observed with rituximab, a long-lasting (at least 6 months, the last observation time) decrease of about 35% to 40% occurred with epratuzumab, with no apparent changes in T cells or immunoglobulin levels. As discussed earlier, the attractiveness of CD22 as a molecular target for therapy in SLE extends beyond the capability of epratuzumab to modestly decrease peripheral blood levels of B cells. CD22 is a cell surface receptor that is a member of the sialioadhesion family and an inhibitory co-receptor of BCR [34]. *In vitro* studies demonstrated that epratuzumab binding can induce CD22 phosphorylation [49] , and the current data from this study suggest that epratuzumab could potentially mediate direct pharmacological effects by negatively regulating certain hyperactive B cells. This hypothesis now needs to be tested. Interestingly, over the period of this study, patients clinically improved without clear evidence of reduction in ANA or anti-dsDNA titers. Similar observations have been reported with rituximab [19] , further supporting the hypothesis that targeted therapy impacting the hyperactive B cell compartment may be successful without needing to completely deplete the broader B cell population.